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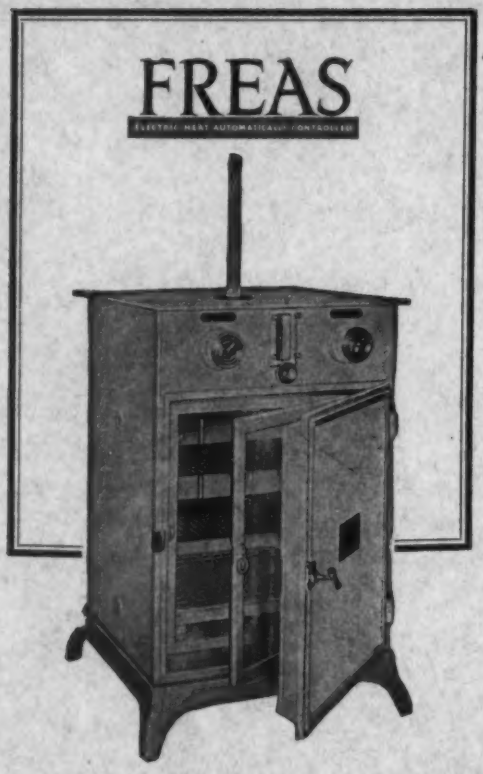

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The Journal of Laboratory and Clinical Medicine

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CLINICAL AND EXPERIMENTAL

THE REACTION OF THE INTESTINAL CONTENTS OF DOGS FED ON DIFFERENT DIETS*

BY W. R. GRAHAM, M.D., RICHMOND, VA., AND E. S. EMERY, JR., M.D., BOSTON, MASS.

THE chemical reaction of the intestinal contents is of interest for a number of reasons. Whether the tract remains essentially acid or alkaline would seem to be of importance from a bacteriologic standpoint. Then there is experimental evidence to suggest that the intestinal musculature may be influenced by the reaction of the chyme. Hammett[1] has shown that the normal intestinal segment shortens, or contracts, with an increase of the hydroxyl-ion concentration; whereas, Young[2] produced the opposite effect, relaxation, with an excess of hydrogen ions. Clinical observation would suggest also that various foodstuffs exert their effects in part by chemical influences, as well as mechanical. Then again, the chemical processes of digestion and absorption may very well be influenced by the chemical reaction of the contents.

LITERATURE

Interest in the chemical reaction of the alimentary tract was manifested several hundred years ago. As early as 1648 van Helmont[3] believed that gastric juice was more than a simple acid, and gastric digestion amounted to more than fermentation alone. For more than two centuries following these views the stomach held the center of the stage, to the almost entire neglect of the intestinal tract. But, in Dunglison's *Human Physiology*[4] of 1838, it is stated that Magendie collected gas from the small intestines of three executed criminals, and had it analyzed. This appears to have supplied the necessary impetus, for there followed numerous, but conflicting, reports upon the reaction of the intestinal contents.

A complete review of the literature upon this subject must necessarily include a great number of references, valuable only in the light of their

*From the Medical Service of the Peter Bent Brigham Hospital.

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historic interest. Therefore, only such studies as are pertinent are included, these for the most part having been published in the light of modern advances in the knowledge of hydrogen ions. No attempt has been made to present references in their chronologic order. But so far as possible, some semblance of anatomic order has been observed, beginning with the duodenum.

For many years it was taught that neutralization of the acid chyme took place within a few inches distal to the pylorus, and that the intestinal reaction remained neutral or nearly so thereafter. There is at present, however, considerable experimental evidence to indicate that this is not always the case; that the duodenum may remain acid throughout its entire length. Grayzel and Miller[5] made hydrogen-ion determinations upon the duodena of thirty-two dogs. These animals were fed on a variety of diets. The nearest approach to alkalinity was found in six dogs on a rachitic diet. The average duodenal P_H of these animals was 6.59. The average of ten dogs on a normal diet was P_H 5.91. The determinations were made both electrometrically and colorimetrically. Long and Fenger[6] studied the reaction of intestinal contents of men. One subject was given an abundant diet of toast, figs, milk, and coffee. The Rehfuss tube was taken soon after breakfast, and allowed to work down through the pylorus. An hour after this the tube was about six inches below the pylorus. Twenty c.c. of practically colorless chyme secured at this point gave a reading, P_H 4.56, in the Hasselbalch cell. On the other hand, McClendon[7] using modern methods, found the adult human duodenal contents to be slightly alkaline, but he does not give the time of removal in relation to the time of eating. An examination of twenty-three samples of duodenal contents from month-old babies, led him to believe that the infant's duodenum is more acid than the average acidity of their stomachs.

In the jejunum, as in the duodenum, the bulk of experimental evidence seems to point to an acid reaction of its contents. McClendon[8] passed a Rehfuss tube in a healthy adult a distance of seven feet from the teeth. The average figures for each day showed the contents to become less acid as the tube descended. The greatest acidity was encountered on the first day, P_H 4.7. The nearest approach to alkalinity was recorded on the fifth day, P_H 6.2. Gold electrodes plated bright with iridium were employed in these determinations. During the same year McClendon, Bissell, Lowe, and Meyer[9] had two other healthy men swallow over seven feet of tubing. The subjects, designated as A and B were fed well balanced diets. Specimens obtained the first day, with both tubes probably in the jejunum, showed a P_H for A, 5.1, for B, 4.5. On the second day A's P_H was 4.9, B's 5.2. During the fourth day several specimens were tested from each of the two tubes. There was a tendency for the material aspirated to become less acid, readings of 6.2 and 6.4 being recorded for B. On the fifth day a specimen from A's tube gave a P_H of 6.5, while B's reading was 5.9. These findings led these observers to express the belief that the alkalinity of intestinal contents increases as one descends from the duodenum toward the ileum. Three years prior to this work Long and Fenger[6] found the contents to be more alkaline. Four male hospital patients, who were not suffering from any disorder of the alimentary tract, consented to swallow Rehfuss tubes for the collection of intestinal speci-

mens. Subject No. 2 swallowed a tube immediately following a meal consisting of meat, potatoes, bread and milk, taken at midnight. At 4 A.M. the tip was some seven or eight centimeters beyond the duodenal-jejunal bend. Collections were then begun and continued, with the following results:

	4:00 A.M.	6:00 A.M.	6:30 A.M	7:15 A.M.	7:45 A.M
P_H	5.36	3.80	5.78	7.30	7.81

Subject No. 3 was allowed a diet of meat, potatoes and bread. Upon the day after the tube had been swallowed the tip had descended well beyond the duodenum. A specimen obtained at this time contained much bile and gave a P_H of 5.62. A day later, the tube had not moved from its position and there was plenty of bile in the specimen obtained. The P_H of this material was 6.26. Subject No. 4 swallowed a tube, and allowed its tip to drop to the beginning of the jejunum before any observations were made. The first liquid brought up some three hours after finishing breakfast showed the presence of bile. Its P_H was 7.69. The same man, one day later, with the tube still in position and bile present, had a P_H of 7.15. These observers appear to place considerable importance upon the presence or absence of bile. Their experience led them to believe if the end of the tube is just below the pylorus, the contents brought up may give a strongly acid reaction; whereas, if at a slightly lower level, the pancreatic juice and bile entering at the same point, may give a distinctly alkaline reaction because of incomplete mixing with the acid chyme. This, of course, may be possible, but they are conditions which McClendon and his collaborators[9] did not encounter. In the upper small intestines of thirty-two dogs Grayzel and Miller[5] found no contents more alkaline than P_H 6.81. The average reading for ten dogs on normal diets was P_H 5.99.

On account of the relative frequency with which fistulae are encountered in the lower levels of the small intestines, the ileum early became a center for observations. In 1879 Ewald[10] studied the material which issued from a fistula, probably situated in the lower third of the small intestine. The fluid had a slightly fecal smell. Its reaction was neutral or faintly acid, never alkaline. Two years later MacFayden, Nencki and Sieber[11] obtained material which was continuously voided through a fistula of the lower ileum. This material was also acid in reaction. These workers, unfortunately, were unable to state their results in terms of P_H. In 1917 McClendon, Shedlov and Thomson[12] selected seven puppies, all from the same litter, for ileal study. The dogs were killed on certain arbitrarily selected days, and determinations were made with the hydrogen electrodes. Their findings were as follows:

AGE DAYS	4	9	11	16	18	42	46
P_H OF ILEUM	5.7	6.75	6.34	6.3	6.1	6.15	6.0

One year later McClendon, Shedlov and Karpman[13] made similar observations upon adult dogs fed on a mixed diet of cooked food. The animals were killed at various intervals after feeding. The hydrogen electrode was inserted through punctures in the intestinal wall, and samples of intestinal contents drawn directly into them. Here is a reproduction of their findings:

DOG NO.	HOUR	P_H	REMARKS
1.	2¾	6.3	First part of ileum
		6.3	" " " " (duplicated)
2.	3½	5.6	" " " "
		5.7	Middle " " "
3.	4	5.7	" " " " $MgSO_4$
		5.6	" " " " "
4.	4½	6.4	First " " " "
		6.6	Last " " " "

Grayzel and Miller[5] in April, 1927, reported similar findings in the lower small intestine. Their table will be reproduced in detail in a later section.

In spite of the ease with which colonic material may be obtained there is an astonishing scarcity of available data upon the reaction of its contents. In 1892 Jakowski[14] reported a case of anus preternaturalis from which acid material issued. Howe and Hawk[15] during 1912 made hydrogen-ion determinations upon the feces of three men in a series of water drinking experiments. They found the reaction uniformly alkaline, the P_H varied between 0.15×10^{-8}, and 9.8×10^{-8}. Water drinking with meals had a tendency to increase the P_H. Pronounced changes in the dietary régime, such as high protein, low protein, and fasting did not affect the P_H sufficiently to cause other than small variations. Jephcott and Bacharach[16] found that albino rats, on certain high calcium low phosphorus diets, developed marked fecal alkalinity. Cod liver oil and irradiated cholesterol restored the feces to acidity. Ultraviolet irradiations had a like effect. Grayzel and Miller[5] reported ten dogs on normal diets. They gave an average cecal P_H of 6.57. The remainder of the colon in the same dogs was P_H 6.84. These authors found that dogs, like rats, produced alkaline feces while on a rachitic diet.

This work suggests that the kind of food eaten, other than a rachitogenic diet, does not influence the P_H. Yet there are conflicting views on this point. Moore and Bergin[17] (1899) placed fistulae in dogs immediately above the ileocecal valve. The animals were fed on lean meat for over three weeks. Daily determinations were made upon the material escaping from the fistulae. It was uniformly found to be alkaline to methyl orange, lacmoid, and litmus, and acid to phenolphthalein. But as the P_H ranges of methyl orange, lacmoid, litmus, and phenolphthalein are: 3.1 to 4.4, 4.4 to 6.2, 4.5 to 8.3 and 8.3 to 10.0[18], respectively, this simply means the P_H of the material was between 6.2 and 8.3. Two years earlier, Moore and Rockwood[19] using litmus papers, litmus solutions and methyl orange as indicators, concluded that the alkalinity of the intestinal contents is greater in herbivora than in carnivora, but feeding carnivora on carbohydrates would greatly increase the alkalinity. They further expressed the belief that fats induce an acid reaction, and that different classes of foods produce different degrees of alkalinity. In contrast to these views, it is interesting to note that McClendon, Meyers, Culligan and Gydesen[20] two decades later, and using modern methods of hydrogen-ion determination, felt that the more soluoble carbohydrate there is present in the food of cats the more acid the intestine may become.

The literature agrees in a general way that the intestinal contents are acid in the duodenum, and become progressively less acid on descending the tract. However, there has been little attempt to study the hydrogen-ion concentration under controlled conditions. Differences in the reports are, therefore, difficult to evaluate. This is particularly true of the work which has been done to show the effect of different foods upon the reaction; then again, the literature published before it was possible to determine the hydrogen-ion concentration is of little value for accurate work. It, therefore, seemed necessary to determine the hydrogen-ion concentration under known conditions, before it was possible to study the various elements that might affect it. Since we started this work, Grayzel and Miller published the results of an almost similar study, but as we were well along on our problem it seemed worth while to complete it.

EXPERIMENTAL WORK

Adult male dogs, with one exception, were selected as the animals of choice for the present work. Dogs were chosen, as their alimentary tract seems to simulate the human as closely as that of any animal, as well as for the ease with which these animals can be induced to take different kinds of foods. It was thought wise to use males to eliminate the possibility of pregnancy or estrus complicating the tests. Adult dogs were used because they are more stable than puppies, and it was thought they might be more apt to eat the various diets. The animals were healthy, and weighed between sixteen and twenty-three kilograms.

Two animals were fed on a mixed diet of cooked horse meat and white bread. The meat contained only a relatively small proportion of fat. Each dog was given approximately 350 gm. of meat and 100 gm. of bread daily. Water was allowed ad lib. This constitutes what will hereafter be termed the normal diet. Dog 1 had been on the above régime for several months. Dog 2 was fed the normal diet for two weeks. In addition, the tract of Dog 3, a female, was studied and included, as it was essentially normal, although having had both kidneys treated with x-rays some time before. The kidneys were delivered through a flank incision, and the surrounding tissues well screened with lead plates so that the rest of the body was entirely shielded. This animal received one-half to one pint of milk daily with the normal diet. She had received this diet for a period of several months. Dogs 4 and 5 had been given approximately 350 gm. each of cooked horse meat daily for fifteen days. This, with water, constituted their entire diet, and will be referred to as the protein diet. Dogs 6 and 7 were given approximately 200 gm. each of white bread, daily, for two weeks. Bread and water was all they received during this period, and constitutes the carbohydrate diet. Dogs 8 and 9 were put upon a diet of fat containing 200 gm. of lard. They were also given 100 gm. of bread to prevent acidosis. On the morning of the twelfth day of this régime animal 8 refused to take his food, and was killed that afternoon. Dog 9, on the other hand, took his daily ration readily, and with apparently much relish. He was killed after having remained on the diet for fourteen days. Except for the bread diet the food

was obviously not limited to one food product. But this never occurs in the diets of humans. A marked preponderance of one type of foodstuff, therefore, satisfied the conditions which were to be investigated.

All except Dogs 1 and 3 were killed approximately twenty-four hours after a feeding. Other workers on this subject apparently have killed their animals or obtained their specimens within six hours posteibum, and for the most part, material has been obtained within one to four hours. McClendon and Sharp[21] in 1919, showed that most foods have an acid reaction, and in general they become slightly more acid on boiling. They found that crushed rabbit muscle gave a P_H of 6.0. The present work was undertaken in an attempt to determine if a permanent change could be produced in the reaction of the intestinal contents by the feeding of protein, fat, or carbohydrate. In order to minimize the effect of the food itself, and at the same time observe a more permanent state of the intestinal contents, it was decided to kill the animals on the day after a feeding, and this was done except for the female (Dog 3) which was killed approximately four hours P. C. Dog 1 was also killed about four hours after a feeding.

The dogs were killed practically instantaneously by intravenous injections of hydrocyanic acid or ether. Dogs 1, 2, and 7 were killed by injecting approximately 15 c.c. of 2 per cent hydrocyanic acid into a hind leg vein. The others were given approximately 20 c.c. of ether in a hind leg vein. Cessation of respiration occurred almost instantly when the full 20 c.c. of ether were injected.

In most instances, the animal's heart was still beating when the abdomen was opened. Various intestinal levels were selected, and immediately tied off between double ligatures to prevent the movement of contents from one portion of the gut to another. Then, the entire intestinal tract, from pylorus to lower rectum, was removed in one piece and washed with tap water. The location of the ileocecal valve was estimated, and a point on the external intestinal wall directly over it chosen as a center from which to measure the distances to the variously selected segments. These measurements were recorded as so many centimeters oral or aboral to the valve. Punctures were then made into the intestinal lumen of each previously selected segment, and the intestinal contents expressed into test tubes containing water of known P_H. It was found impractical to obtain the material under oil. Whenever possible, two specimens were obtained from the same segment, for duplicate determinations. Within a period varying between ten minutes and one and one-half hours, the material was transferred from the test tubes to centrifuge tubes, centrifuged, and the comparatively clear top fluid decanted into the reading tubes and tested colorimetrically.

The hydrogen-ion concentration was determined colorimetrically, as this method gives sufficiently accurate readings for the work in hand. (See Grayzel and Miller.[5])

DISCUSSION OF RESULTS

Table I shows quite clearly that no fundamental changes in reaction occurred with a protein, fat, or carbohydrate diet. This can probably be more readily seen in Chart I. These curves portray graphically the result ob-

tained when the average readings for dogs on the same diet are plotted, using P_H as ordinates and per cent distance from pylorus to ileocecal valve as abscissae. The numerals 1, 2, 3, etc., represent the dog number; e.g., 4 and 5 were both on a protein diet, while 8 and 9 received one consisting chiefly of fat. With the exception of 2 (dog on normal diet killed twenty-

TABLE I

DOG NO.	DIET	KILLED	DUODENUM	UPPER-SMALL INTESTINE	MIDDLE-SMALL INTESTINE	LOWER-SMALL INTESTINE	CECUM	COLON
1	Normal	4 hr. P. C.*	5.3 P.	6.0 P.	6.6 P.	7.0 P.	No spec.	6.1
2	"	24 " "	6.6 M.	6.7 S.	6.7 S.	7.2 S.	6.8	6.2
3	"	4 " "	6.0 P.	6.0 P.	6.1 P.	6.9 P.	5.8	No spec.
4	Protein	24 " "	5.9 P.	6.1 P.	6.5 P.	6.5 P.	6.0	6.6
5	"	24 " "	6.5 M.	6.3 M.	6.6 S.	6.5 S.	6.6	6.6
6	Carbohydrate	24 " "	6.3 M.	6.6 M.	6.4 M.	7.2 S.	6.4	6.8
7	"	24 " "	6.3 M.	6.5 M.	6.7 S.	7.1 S.	6.8	No spec.
8	Fat	24 " "	6.4 M.	6.4 P.	6.6 P.	6.7 P.	6.4	6.4
9	"	24 " "	6.3 M.	6.3 P.	6.5 P.	6.0 P.	6.2	6.3

*P. C.—Postcibum.
P.—Plenty of contents.
M.—Moderate amount of contents.
S.—Small amount of contents.

four hours postcibum), the curves are very well bunched, no great fluctuations occurring. It will be noticed that at 10 per cent of the distance from the pylorus to the ileocecal valve there is a difference of less than 0.2 between the highest and lowest P_H values of all curves, except 2. At 50 per cent of the distance the maximum difference of any two readings is less than 0.1. From this point down the tract there is a gradual decrease in acidity, the highest point being reached between 80 and 95 per cent of the distance from pylorus to cecum. Curves 4-5 (protein diet) and 8-9 (fat diet) reached their height at 80 while 1-2 (normal diet) and 6-7 (carbohydrate diet) attain their maximum at 95 per cent. It is our feeling that no particular significance should be attached to the more alkaline readings in the lower ileum for carbohydrate and normal diets as compared with the readings on the other diets. This is thought to be due to minor variations that will be discussed later, and that it is only the result of chance that it occurred this way. Only curves 6-7 and 2 really extend beyond neutrality. We cannot explain the greater alkalinity found in curve 2 over that of 1-3 which represents a normal diet. At first thought it might be due to the fact that 2 was killed twenty-four hours after feeding, whereas 1 and 3 were killed within six hours after feeding. But on the basis of this explanation one would expect that 2 would more closely approach the other animals killed after a day's fast and curves 1 and 3 would be isolated, which is not the case. In all instances both cecum and colon were more acid than the ileum. There is a general tendency for the cecal content to be more acid than that found farther along the colon.

The outstanding feature of Chart I is the general type of curve, being acid in the duodenum, becoming gradually less acid on descending the tract,

reaching neutrality or even alkalinity in certain instances, at the end of the ileum. The curve then takes a sudden drop to acidity in the cecum. Although the curves are not smooth it is felt that the notchings would disappear if more animals were studied and averaged. Compare Charts I and II for this purpose. There is no evidence from this work, therefore, to suggest that different foodstuffs will permanently change the hydrogen-ion concentration in the tract. It suggests that if feeding certain food substances tends to change the flora, and a change in flora influences the response of the tract, that it does not do so through influencing the P_H of the small intestine.

It seems that the reaction of the intestinal contents should depend on the relative proportions of its ingredients: foods, saliva, gastric juice, pancreatic juice, bile, and succus entericus. As already stated, McClendon and Sharp[21]

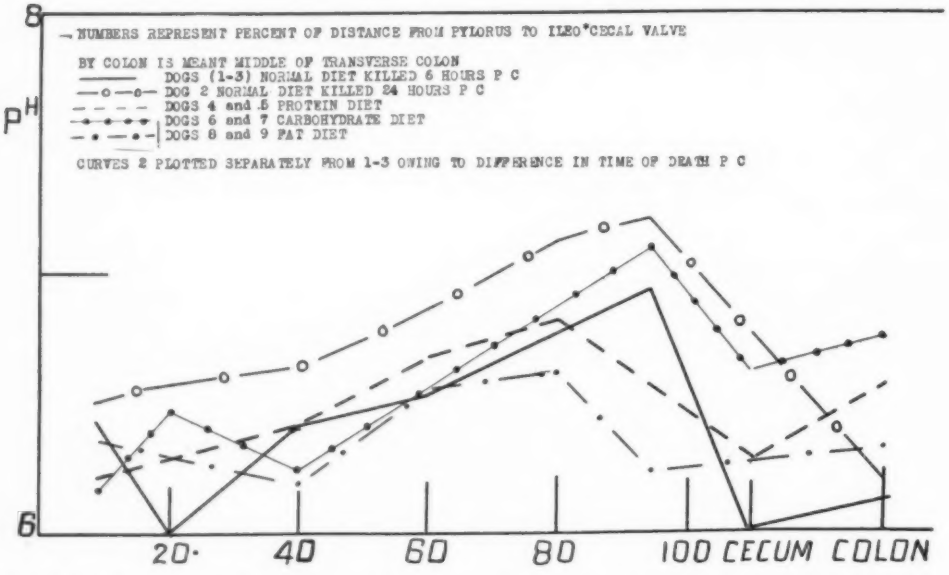
8
6
pH
NUMBERS REPRESENT PERCENT OF DISTANCE FROM PYLORUS TO ILEO-CECAL VALVE
BY COLON IS MEANT MIDDLE OF TRANSVERSE COLON
DOGS (1-3) NORMAL DIET KILLED 6 HOURS P C
DOG 2 NORMAL DIET KILLED 24 HOURS P C
DOGS 4 and 5 PROTEIN DIET
DOGS 6 and 7 CARBOHYDRATE DIET
DOGS 8 and 9 FAT DIET
CURVES 2 PLOTTED SEPARATELY FROM 1-3 OWING TO DIFFERENCE IN TIME OF DEATH P C
20
40
60
80
100 CECUM COLON

Chart I.—The average P_H of intestinal contents of dogs at different levels. Note: Abscissa represents per cent of distance from pylorus to cecum.

showed that most foods have an acid reaction. Saliva is usually slightly alkaline, but gives such a faint reaction in either direction that it may be neglected. Gastric juice is, of course, normally quite acid, being equivalent to 0.46 to 0.58 per cent hydrochloric acid. Starling,[22] in his *Principles of Human Physiology*, states that pancreatic juice is markedly alkaline in reaction, varying between N/10 and N/7 sodium carbonate. He further states: "It is therefore about as alkaline as gastric juice is acid, and it will be found that equal quantities of gastric juice and pancreatic juice when added together practically neutralize one another." During 1914, Drury, McMaster and Rous[23] while studying the relation of the reaction of the bile to experimental cholelithiasis found the normal liver bile to be alkaline in reaction, with an average P_H of 8.20. But in the gall bladder, the bile becomes on long sojourn there strongly acid, its P_H ranging between 5.18 and 6.00. Our own

determinations on specimens from the gall bladders of five dogs were somewhat higher than those given above, showing an average of P_H 6.56. Both Howell[24] and Starling[22] agree that the succus entericus is distinctly alkaline in reaction. It is therefore not surprising to find that the upper intestinal levels are most acid, becoming gradually less and less, as absorption takes place, with a maximum of alkalinity in the terminal ileum.

Let us consider in more detail the mechanism involved. Food mixed with acid gastric juice enters the duodenum. Here it comes in contact with at least slightly acid bile and pancreatic juice which is not quite so alkaline as gastric juice is acid. Therefore, due to the excess of acidity of bile and gastric juice over the alkalinity of the pancreatic secretion, one should expect to find the upper intestinal tract definitely acid in reaction. But as the intestinal contents move down the tract, undergoing digestion and absorption and

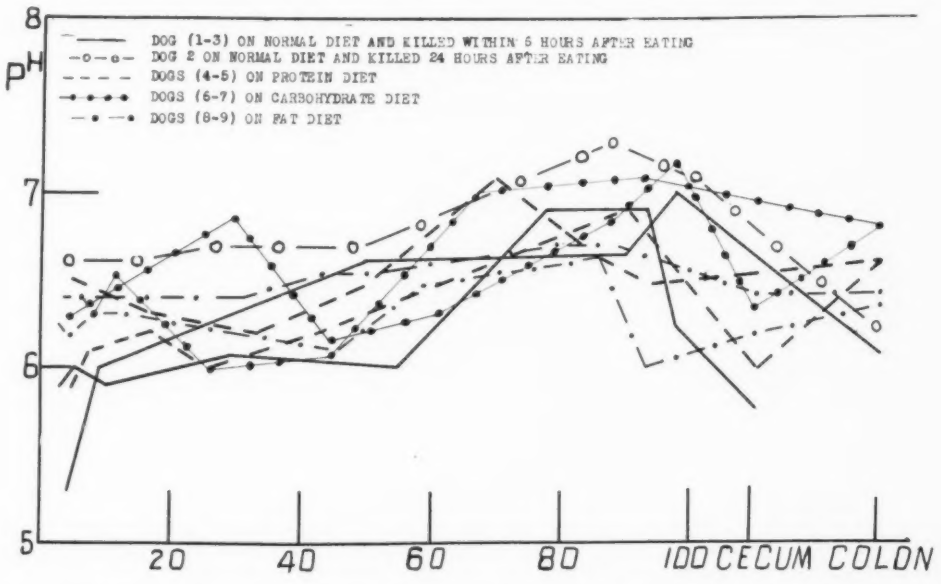

Chart II.—Curves to show fluctuations in the P_H of the intestinal contents at different levels. Note: Abscissa represents per cent of distance from pylorus to cecum.

mixing with the distinctly alkaline succus entericus, it is only reasonable to expect the content to become less and less acid. On this hypothesis, material which reaches the terminal ileum should contain relatively less of the acid elements and more of the alkaline ones than at any higher level. By this sort of mechanism we are able to account for the alkaline content observed in the last 15 to 20 per cent of the distance from pylorus to cecum.

Chart II shows quite strikingly the fluctuations which may occur in consecutive intestinal segments. Here again, the numerals, 1, 2, 3, etc., refer to the animal number. Probably the most prominent feature here is the lack of smoothness of the curves. At the time of studying the intestine, it was noticed that segments would vary a good bit in the amount of material they contained. In most instances the contents would be more alkaline from comparatively empty segments than from full ones. This was really more strik-

ing than Chart II would indicate and was noticeable enough for one of us to try and predict whether the readings would be higher or lower than those for the contents of the surrounding segments.

As regards the terminal ileum one expects high readings from the comparatively excessive amount of succus entericus, irrespective of the quantity of the material obtained.

One other point may be of passing interest. For the most part the P_H of the upper tract approaches fairly closely to the P_H of bile. Starling's[22] observation that the concentration of the pancreatic and gastric secretions are such as might tend to nullify each other, lends some support to the possibility that the bile may be the dominating factor in the intestinal acidity.

Clinically there is reason to feel that foods affect the intestinal tract chemically. With the possible exception of the dogs fed with fat, there is no

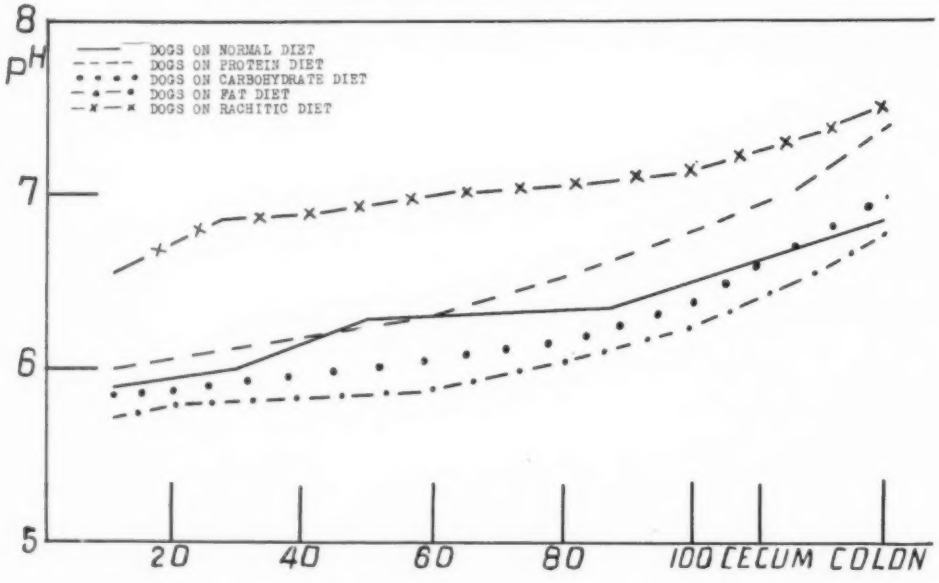

Chart III.—Curves plotted from the published table of Grayzel and Miller for comparison with our results. Note: Numbers only approximate. The authors not having given measurements.

evidence to feel that the other foods fed affected even temporarily the P_H. It is not likely that the slightly acid reaction which McClendon[21] has found most foods possess, would have much influence by itself. It is conceivable, however, that foods may influence the P_H by the relative amounts of the different secretions they call forth. Thus fat is known to inhibit hydrochloric acid and stimulate the contraction of the gall bladder. The curves obtained from the dogs fed on fat show a tendency to more acid readings in the ileum than in the case of the other dogs. These dogs were fed excessive quantities of fat, and the intestines were found to contain large amounts of fatty material throughout their entire length. The tendency toward a more acid reaction may, therefore, be due to the fatty acids produced.

The finding of acid contents in the cecum was unexpected. But in every case the cecal contents had a lower P_H than the ileum. It is interesting to

contrast our results from this point of view with those of Grayzel and Miller.[5] They have done essentially the same thing as we did. Having studied the effect of different kinds of food products on normal dogs they determined the effect of feeding a rachitic diet. In dogs on a rachitic diet the P_H was considerably higher than on a normal diet. We have taken the liberty of charting the results from their table in the same general way as our own for the sake of making comparison simpler. Their curves are smoother than ours, but agree with ours in the gradual sloping up grade from the acid duodenum to the more alkaline ileum. However, our curves show a greater tendency to alkalinity in the ileum than any of theirs except that obtained on a rachitic diet. This might be explained by their having killed the dogs within a few hours postcibum; whereas, ours for the most part were killed after twenty-four hours. On the other hand the most alkaline reaction in our series occurred in a dog on a normal diet which was killed within six hours. The striking difference between our results are the findings in the large intestine. Their results show a more alkaline colon than ileum; whereas, the colon of our dogs invariably gave a more acid reaction than in the lower ileum. We are not able to explain these divergent results.

SUMMARY AND CONCLUSIONS

Previous work done on the reaction of the intestinal contents has been unsatisfactory in that much of it was done before accurate determinations of hydrogen-ion concentration could be made, and because of a lack of controlled conditions.

The present study was undertaken to determine the hydrogen-ion concentration of all portions of the tract simultaneously, and to determine whether the feeding of a preponderance of one type of food product would change the reaction.

Three dogs were fed diets considered normal for these animals. The others were given a preponderance of protein, fat, or carbohydrate for two weeks, and then killed instantaneously twenty-four hours after eating and the tract studied immediately.

All showed about the same thing. The P_H ranged from 6.2 to 6.5 in the duodenum. Thence it became gradually more alkaline as it approached the ileocecal valve to become more acid in the cecum. This agrees for the most part with the results obtained by Grayzel and Miller[5] except that they found the contents of the cecum to be more alkaline than in the terminal ileum. We have no explanation for these divergent results.

Our results suggest that the reaction of the upper intestine is acid as a result of the hydrochloric acid and bile, and become less so as the alkaline succus entericus becomes the more dominant factor. There is no evidence from our results that two weeks' feedings of different food substances influence the P_H of the intestinal tract.

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THE CLINICAL SIGNIFICANCE OF EOSINOPHILIA ON A GENERAL MEDICAL SERVICE*

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REVIEW OF LITERATURE

IT HAS seemed to us that textbooks in their discussions of eosinophilia have been content to list the conditions in which an increase in eosinophiles *may* occur without giving much idea about their actual occurrence. The present survey was undertaken, therefore, to determine, if possible, the clinical significance of eosinophilia among the population of a general medical service.

It is proposed first to quickly scan some of the more outstanding features of the eosinophile with such references as may render the original literature easy of access.

In 1864, Wharton Jones[1] first distinguished finely and coarsely granular leucocytes in fresh blood, some of the coarsely granular variety being subsequently shown by Ehrlich[2] to have specific affinity for eosin, since which time the so-called eosinophiles have assumed increasing importance. The subject in general has been quite extensively reviewed by Opie,[3] Brown,[4] Herrick,[5] Simon,[6] Schmite,[7] Ewing,[8] Schambert and Strickler,[9] French,[10] Stäubli[11] and Zappert.[43]

The eosinophile is common to all mammalian species.[3] Most hematologists are agreed as to the origin of eosinophiles from cells of a lymphoid character. The granules of the eosinophiles derived from the bone marrow are of true endogenous origin. They are differentiated gradually from a basophile protoplasm.[12] Numerous investigators have described local heteroplastic development of eosinophiles in various regions in the body.[13]

The average number of eosinophiles in the blood of man is from 2 to 4 per cent, but it has been shown to vary significantly from hour to hour.[14] They are more variable in the blood of children and usually higher in number than in the adult. Six per cent is considered by many a fair average for the normal child.[15, 43, 73] Variations in weight of the body seem to cause marked changes in number at least in the guinea pig.[3] Fall in weight invariably seems to produce an increase in eosinophiles. There may be cases of so-called "constitutional eosinophilia" in which the eosinophiles reach as high a value as 66 per cent in an otherwise apparently healthy man. These cells may also be high in the patient's blood relations.[16] At present we are unable to generalize as to the mechanism of the mobilization of eosinophiles. One of the most useful theories states that anaphylaxis and eosinophilia are usually coincident.[34, 68, 69, 70] Actually this is often found to be the case.

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In diseases of parasitic origin, especially the animal parasites, eosinophilia is the rule. Oxyuris,[17] Ascaris,[18, 19] Taenia saginata,[20] Echinococcus,[21, 22] Trichina,[23, 24, 67] Uncinaria,[25, 26] Filaria bancrofti,[27, 28, 28a] Bilharzia,[29] Dibothriocephalus and Taenia solium[30] usually show moderate eosinophilias (8 to 20 per cent). Many others show inconstant eosinophilia such as the Leishmania and Trypanosomes. Amebic dysentery often produces an increase in eosinophiles whereas in the bacillary type they decrease in number.[31] Many of the protozoa and some of the cestodes do not show an increase in the eosinophile count.

Some skin diseases induce eosinophilia, especially dermatitis herpetiformis, bullous dermatitis, pemphigus, scabies, mycosis fungoides, herpes iris, erythema bullosum, psoriasis and prurigo.[9, 32] In eczema it is only occasionally found.[9]

Asthma and hay fever typically bring about a very definite increase in eosinophiles.[17, 33, 34, 35, 43, 72, 75] Rheumatic fever commonly shows an increase,[36, 37] migraine at times,[38] also Quincke's edema,[39] and serum sickness.[75]

It has been repeatedly proved that foreign proteins, such as egg albumin,[40, 41, 42] horse serum[40, 43] and tuberculin[43] produce eosinophilia.

Chronic bronchitis with emphysema at times may be differentiated from asthma on the basis of the eosinophile count inasmuch as the former seldom induces any marked increase in their number.[8]

Scarlet fever seems to be the only acute exanthem in which a marked eosinophilia is found. Eosinophilia is believed to be a favorable sign, disappearance or a marked fall indicating a serious prognosis.[44, 45] In diphtheria, eosinophiles are not ordinarily increased unless a foreign protein is introduced, such as antitoxin.

In the case of tuberculosis, especially of the intestinal variety, there may be a marked eosinophilia;[15] however, in tuberculosis of the lung an absence of eosinophilia is often seen. With suppuration and cachexia they may reappear.[8] There may be a high eosinophile count in leprosy.[46, 47] It is claimed that paratyphoid B may be differentiated from typhoid by the presence of eosinophiles in the former disease.[48] In general, it can be said that following most infections with a return of the leucocyte count to normal, there may be a moderate rise in the eosinophiles—the so-called "reactive" eosinophilia. In nearly all forms of acute polynuclear leucocytosis an absence of eosinophiles is found. Scarlet fever, acute rheumatic fever, and malaria at times furnish notable examples.

Malignant tumors rarely produce a high eosinophile count.[8, 49, 50] Tumors of the colon[71] and cervix[51] may produce both local and general eosinophilia.

The proportions of eosinophiles in leucemia are generally within normal limits, but their total number is greatly increased. Eosinophilic leucemias have been reported showing remarkably high counts.[52, 53, 54, 55, 56] Splenomegaly seems very usually associated with this type of leucemia. In some cases of Hodgkin's disease, the eosinophiles may reach a high level.[57, 58, 59]

Local eosinophilas have been quite extensively reported, especially pleural,[60, 61, 62] intestinal,[63, 64, 65] and skin exudates.[66] Rickets, osteomyelitis, osteo-

malacia, malaria, Addison's disease, gonorrhea, varicella, chlorosis, echinococcus disease, Hodgkin's disease and uremia occasionally create an eosinophilia.

It is interesting to note that under the liver treatment for pernicious anemia there is a progressive rise in the eosinophile count.[74]

CLINICAL DATA

The unit histories of 13,340 patients who entered the Presbyterian Hospital between March 30, 1923, and October 12, 1927, were examined. Of these, approximately 40 per cent, or about 5,500 patients, were medical cases and these form the basis of the present report. All these cases had at least one complete blood count including a differential leucocyte count from a stained smear. Furthermore, on admission, stool examination including a search for ova and parasites was done in practically all cases routinely. In case an eosinophilia was discovered in the blood, several careful searches for intestinal parasites or their eggs were usually made.

Two factors prevent this survey from being entirely representative; no cases of the acute exanthemata are admitted to the hospital, and few dermatologic cases are seen. Moreover, it is entirely possible that occasional increases in the eosinophiles that are more or less transitory may have been missed by too infrequent counts, especially the so-called "reactive" eosinophilias occurring in postfebrile states after the return of the total leucocyte count to normal.

By an eosinophilia we mean a condition in which there is 5 per cent or more of eosinophiles in the blood, thereby accepting 4 per cent as the upper limit of normal. The hopelessness of such an arbitrary division between the significant and insignificant is obvious. However, in the facts presented below, we shall endeavor to point out those counts that might be misleading, i.e., those in which the eosinophiles are only 5 per cent, but which are included in our list as eosinophilias.

Diseases of the blood, such as anemias, leucemias, purpura, and hemophilia were excluded from this summary.

PARASITIC INFECTION

There were 31 cases due to infection with parasites. Of these, 15 were cases of trichiniasis. In this condition, as is well known, eosinophilia occurs with great constancy. Eosinophiles may form the majority of the circulating leucocytes reaching as high as 81 per cent in one of our cases, while in only one case was the count constantly below 15 per cent. It is interesting to note that the total white count is not necessarily high, for in 6 of this series, the leucocytes were less than 10,000 per c.mm. In general the total count averaged 12,000 to 15,000 with 20, 30, or 40 per cent eosinophiles. Occasional counts were recorded as high as 28,000 and 32,000.

A diagnosis of amebic dysentery was made in 5 cases. In these, the eosinophiles were low (5, 5, 6, and 7 per cent), with the exception of one case in which the eosinophiles ranged from 14 to 62 per cent.

A miscellaneous group of 11 cases included: ascaris, two (11 per cent, 19

to 22 per cent eosinophiles); diboth riocephalus latus, three (5 per cent, 6 per cent, and 4 to 17 per cent); uncinariasis, usually with trichuris trichura, three (10 per cent, 13 to 24 per cent, 10 to 33 per cent); taenia saginata, one (8 per cent); oxyuris vermicularis, one (7 per cent); filariasis, two (9 per cent, 10 to 12 per cent).

DISEASES OF LUNGS AND UPPER RESPIRATORY TRACT

Sinusitis.—Three cases diagnosed as sinusitis showed eosinophilias of 5 per cent, 8 per cent, and 8 per cent respectively. The total leucocytes were normal in all cases. Four additional cases of sinusitis, three in connection with asthma and one with a generalized acute upper respiratory infection had an increase in eosinophiles.

Acute Pharyngitis, Tonsillitis, Bronchitis, etc.—There were 13 cases. Eosinophilia was rarely striking (5 per cent—4 cases; 6 per cent—4 cases; 7 per cent—2 cases; 9 per cent—2 cases). In one case of acute follicular tonsillitis, the eosinophiles were on one occasion 15 per cent and on another 18 per cent.

Lobar Pneumonia.—Seven cases. Eosinophiles ranged from 5 per cent to 7 per cent. The eosinophilia occurred in every case as a "reactive" phenomenon, i.e., with a return of the leucocytes to lower or normal levels.

Bronchopneumonia.—Six cases. Range 5 per cent to 8 per cent. In this condition, eosinophilia was less often a "reactive" phenomenon and in the majority of cases occurred at the height of the disease.

Asthma, Chronic Bronchitis, Emphysema and Bronchiectasis.—Thirty-eight cases. The diagnosis of asthma alone was made in 17 cases. In these cases the eosinophiles ranged from 5 per cent to 18 per cent, averaging 10 per cent. One interesting case was that of an asthmatic with 6 per cent eosinophiles who had a bout of acute rheumatic fever. With the accompanying leucocytosis the eosinophiles dropped to 1 per cent and later rose again with the return to normal leucocyte count. The diagnosis of asthma occurred in combination with others of the group in 11 cases.

The diagnosis of chronic bronchitis alone was made in two cases (5 per cent, 6 per cent) and with others in the group in 10 cases (range: 5 to 13 per cent; average, 8 per cent). In one case chronic bronchitis and syphilis were associated.

Emphysema was diagnosed alone in two cases (5 per cent and 10 per cent) and in combination in 7 cases (6 per cent to 9 per cent).

Bronchiectasis occurred in 5 cases: once alone (5 per cent), twice with others of this group (5 per cent and 7 per cent) and twice in lueties (5 per cent and 6 to 11 per cent).

Chronic Tonsillitis.—In 3 cases so diagnosed the eosinophiles were 5 per cent, 5 per cent, and 9 per cent.

Serofibrinous Pleurisy.—Five cases (5, 6, 8, 8, and 13 per cent). In one case the patient was luetic.

Pulmonary Tuberculosis.—Five cases occurred with eosinophiles of 5 or 6 per cent. In two additional cases (6 per cent and 8 per cent) tuberculosis and syphilis were associated.

RHEUMATIC FEVER GROUP

In this group are included cases of acute and chronic rheumatic fever, chorea and rheumatic heart disease. There were 40 cases. Two of these patients had erythema multiforme and one case was luetic. Eosinophilia was inconstant even in the same patient and did not seem to have a definite relation to the total leucocyte count although in some cases it was definitely of the "reactive" type. The usual degree of eosinophilia was from 5 to 9 per cent. Six cases were 10 per cent or above, the highest being 19 per cent.

In one remarkable case there were no eosinophiles until the development of a skin lesion suggesting pellagra, whereupon the eosinophiles rose rapidly to 12 per cent to 24 per cent and to 40 per cent associated with a rise in the total leucocyte count to 19,000. Six weeks later the leucocytes were 11,000 and the eosinophiles 3 per cent and sometime later 19,000 and 12 per cent.

"DEGENERATIVE" DISEASES

In this group are included chronic multiple arthritis, general arteriosclerosis, chronic nephritis, and hypertension. No attempt has been made to further subdivide this group. These were 28 cases. In this group are included 3 cases who had also chronic cholecystitis, one with syphilis, one with hay fever, and three with diabetes. As a rule the eosinophilia was not high—about 5 to 8 per cent.

HYPERTHYROIDISM

There were eight cases with a range of from 5 per cent to 9 per cent.

DERMATOLOGIC CASES

This is in no wise a representative group as patients with skin lesions are treated only as out-patients with few exceptions. There were 15 cases in this group. Of these, eight were miscellaneous conditions with eosinophile counts of from 5 to 19 per cent. An interesting case was one of leucoderma, urticaria, chronic bronchitis, and asthma. The patient repeatedly had an eosinophilia of 33 to 36 per cent.

Six cases of arsenic poisoning during antiluetic therapy formed a conspicuous group. The eosinophilia in these cases was 6 per cent, 7 to 9 per cent, 13 per cent, 18 per cent, in one case 24 to 28 per cent, and in the sixth case the eosinophiles reached 65 per cent of the total leucocytes, which numbered 12,000 per c.mm.

SYPHILIS

An eosinophilia of from 5 to 17 per cent was present in ten cases in which the primary diagnosis was syphilis. In addition, syphilis was present in 12 of the above mentioned cases and may have been an important factor.

MISCELLANEOUS

There were 84 cases in this group. In a great variety of conditions represented by only one case, an eosinophilia of 5 per cent or even of 6 per cent occurred, often transiently. We believe that this is casual and of no signifi-

cance and these cases are accordingly not treated in detail here. There were 42 such cases. The remaining 42 may be listed as follows:

TABLE I

DIAGNOSIS	NO. CASES	% EOSIN.	DIAGNOSIS	NO. CASES	% EOSIN.
Chronic Colitis	6	6–11	Carcinoma, liver	2	6–11
Acute Colitis	1	10	Lymphosarcoma	1	7–10
Periarteritis nodosa	2	8–17	Pyelitis	4	5–15
T. B. of male genitals	1	7	Nephrolithiasis	1	6–12
Gastroptosis	1	7	Hem. Staph. abscesses	1	7
Undiagnosed condition of abdomen	1	10	Chronic osteomyelitis	1	9
Ulcer of duodenum	6	5–8	Scarlet fever	1	7
Constipation	1	10	Sprue	1	8–27
Anxiety neurosis	1	8	Lead poisoning	1	5–8
Angioneurotic edema	1	14	"Secondary anemia"	1	8
Idiopathic epilepsy	1	7	Thromboangeitis oblit.	1	10
Paralysis agitans	1	7	Dysmenorrhea	1	
Carcinoma, rectum	1	12	Diag. unknown	1	14–20
			Diag. unknown	1	73

COMMENT

Parasitic infestation accounted for 10 per cent of our cases of eosinophilia and of these half were trichiniasis. This group included some of the highest eosinophile counts, although in amebic dysentery the eosinophilia is uniformly slight. The highest count in our series was in a case of trichiniasis, 81 per cent.

In infections of the respiratory tract occasional cases of uncomplicated sinusitis exhibited persistent eosinophilia. An occasional case of lobar pneumonia showed a "reactive" eosinophilia. Probably a great many such reactions were missed because of their transitory character. In contrast to lobar pneumonia, bronchopneumonia may show an eosinophilia at the height of the disease with a considerable leucocytosis. The chronic pulmonary affections (chronic bronchitis, emphysema and asthma) were well represented in this series accounting for about 13 per cent of the total.

The rather high incidence of eosinophilia in the rheumatic fever group was a surprise to us. There were 40 cases (13 per cent) in this series. The eosinophilia was often very transient in the individual patient and while often of the "reactive" type was by no means constantly so. Further work is in progress by one of us to determine what relation the appearance of an eosinophilia has to the various phases of the disease.

Nearly 10 per cent of the total number were cases of hypertension, chronic nephritis syndrome. The eosinophiles while not striking (usually 5 to 8 per cent) were generally persistent.

Not infrequently an eosinophilia is found in cases of hyperthyroidism, syphilis, chronic colitis, duodenal ulcer, and pyelitis. Eosinophilia is common in arsenic poisoning with skin manifestations.

In fully 40 per cent of the cases in the present series the eosinophilia occurred in such a small percentage of the cases of a given group or came so narrowly within our arbitrary definition of eosinophilia that we deem it of no diagnostic importance.

It will be seen in the above that eosinophilia occurred in the majority of cases in our series in conditions that theoretically, at least, may be related to allergy. The relation of parasite to host, for example, is little understood and may be allergic. In pneumonia a definite antigen-antibody mechanism is at work. Conditions of chronic infection may belong to this group. The recent work of Swift and others tends to place rheumatic fever upon an allergic basis. It is in these conditions that eosinophilia is likely to occur. Its exact relation to allergy is not known and much interesting work remains to be done in this field.

SUMMARY

1. An eosinophilia of 5 per cent or more occurred in 300 patients among 5,500 general medical cases, all of whom had complete blood counts and stool examinations. The small number of dermatologic cases seen and the entire absence of scarlet fever patients prevent this series from being wholly representative. Cases of obvious blood diseases were excluded.

2. Of the 300 cases of eosinophilia in this series, 10 per cent occurred in parasitic infestation; 13 per cent in rheumatic fever; 13 per cent in chronic pulmonary disease (chronic bronchitis, emphysema, asthma); 10 per cent in the chronic nephritis-general arteriosclerotic group. In fully 40 per cent the eosinophilia occurred in isolated cases of various conditions and had no diagnostic significance.

It is suggested from this series that eosinophilia may be a part of the phenomenon of allergy.

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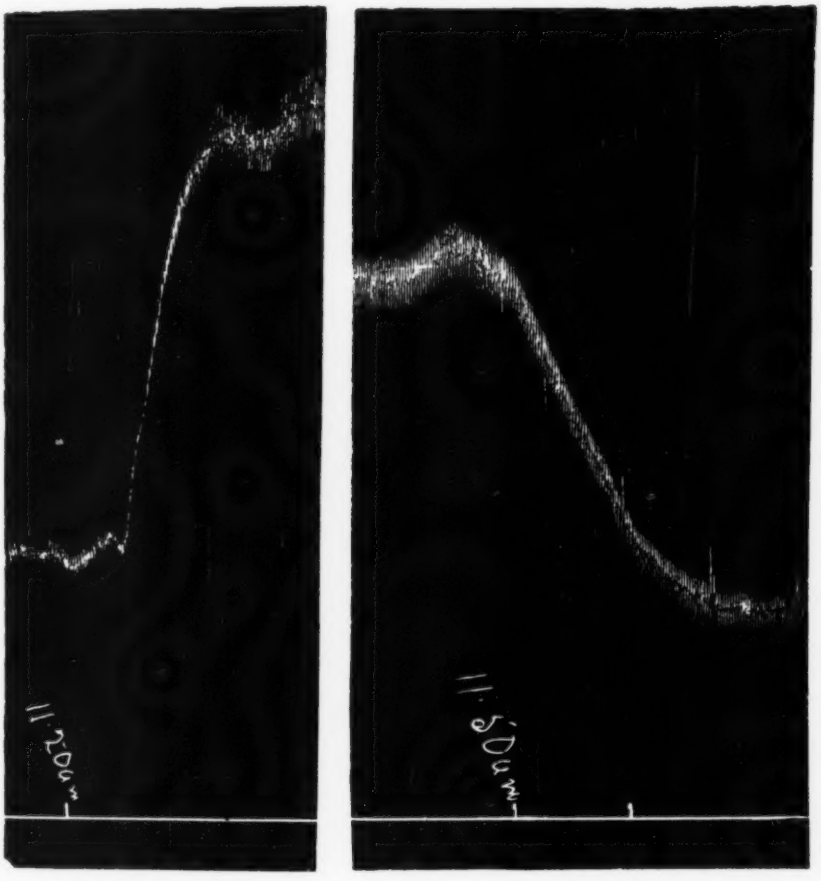
ON THE PHYSIOLOGIC ACTION OF PRESSOR X (COLLIP)*

By S. Soskin, Toronto, Canada

PROFESSOR J. B. COLLIP[1] (1928) has recently prepared extracts containing a pressor substance, from various animal tissues and organs. At his request, we have investigated the physiologic action of several of these ex-



Started injection. Stopped injection.

Fig. 1.—Dog 3. Blood pressure. Three c.c. pressor X testes (1) in 150 c.c. saline intravenously.

tracts, and it seems advisable, for the guidance of others, to place on record a brief statement of the chief results. Like epinephrin, this substance, which one may call pressor X, causes a marked rise in blood pressure accompanied

*From the Department of Physiology, University of Toronto.

[1]Collip, J. B.: Am. Jour. Physiol., 1928, lxxxv, 360.

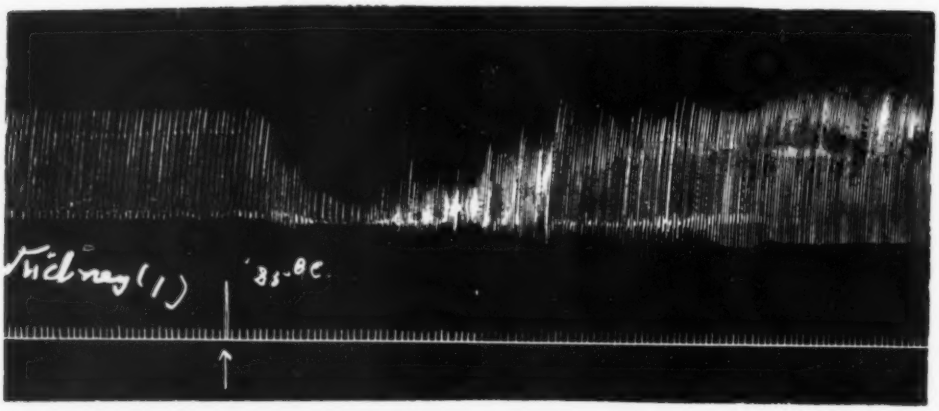


Fig. 2.—Perfused rabbit heart. Pressor X (kidney) added to perfusion fluid.

Fig. 3.—Dog 2. Pressor X intestine.

by an acceleration of the heart rate and a diminution in kidney volume, but its action on the intestine, uterus, and the blood sugar is quite different.

Injected continuously into the veins of dogs, anesthetized with ether or

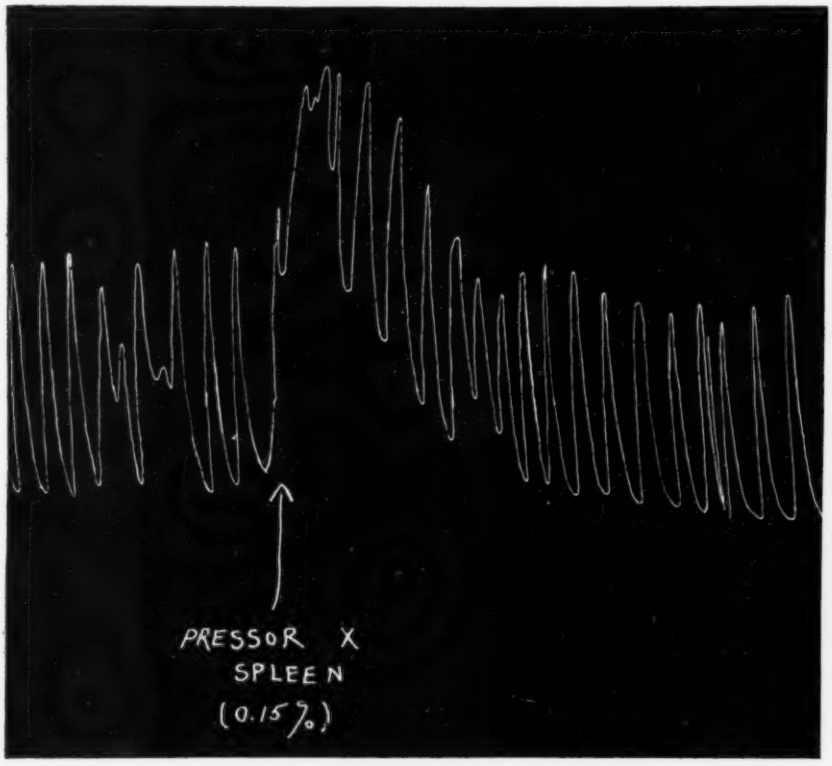


Fig. 4.—Rabbit intestine.

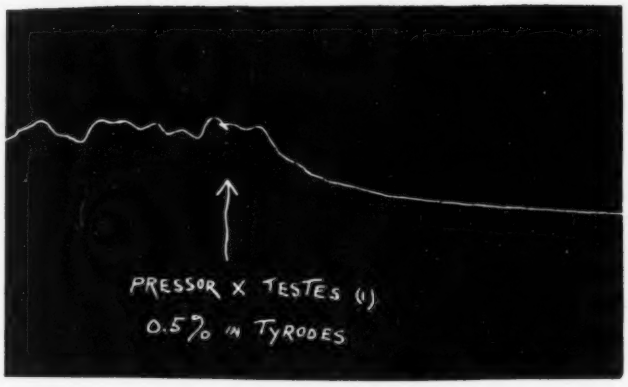


Fig. 5.—Virgin guinea pig uterus.

amytal, the substance caused a marked rise in blood pressure, which fell, to the initial level or below it, immediately the injection was discontinued. (Fig. 1.) The rise in blood pressure was usually accompanied, in the intact animal, by

a slight acceleration of the heart. This acceleration was also observed in the isolated perfused mammalian (rabbit) heart, this being preceded by a slowing and lessening of the beats. (Fig. 2.) With the increased blood pressure there occurred a fall in kidney volume, as shown in Fig. 3. This tracing also shows the initial fall, preceding the rise in blood pressure, due to the presence of a depressor substance (probably histamine). It should be pointed out that this was observed only in extracts of intestine, but not in the other extracts. Intravenous injection of pressor X into decapitate cats produced pressor

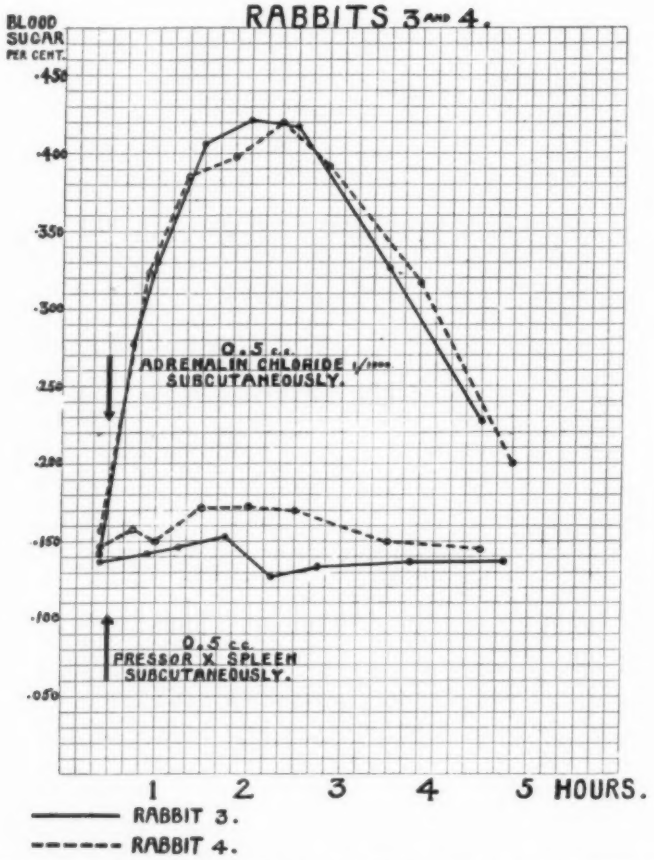


Fig. 6.—This shows the striking difference between pressor X and epinephrin, as regards their effect on the blood sugar. The pressor extract used in this experiment had been previously shown to exert about the same influence on the blood pressure as a 1/1000 solution of adrenalin chloride (Parke Davis).

effects quite similar to those obtained in intact animals. In decapitate eviscerated cats and in an eviscerated dog, it produced a definite rise in blood pressure, though not so marked as in the intact animal, indicating, therefore, a constriction of the blood vessels of the muscles and other extraabdominal tissues.

When added to the nutrient fluid, pressor X caused only a slight and transient increase in the tonus of the isolated rabbit intestine (Fig. 4). It did

TABLE I

RABBIT	EXTRACT	ROUTE	NORMAL BLOOD SUGAR PER CENT	HIGHEST BLOOD SUGAR PER CENT WITHIN 4 HOURS
1	Intestine	Subcutaneous	0.181	0.210
2	"	"	0.153	0.197
3	Spleen	"	0.138	0.153
4	"	"	0.147	0.173
5	Testes	Intravenous	0.182	0.202
6	"	"	0.183	0.177

not revive the contractions inhibited by epinephrin. It inhibited the rhythmic contractions of the similarly observed virgin guinea pig uterus. (Fig. 5.)

Perhaps the most interesting contrast between pressor X and epinephrin is with regard to its effect on the blood-sugar level. Compared to the effects

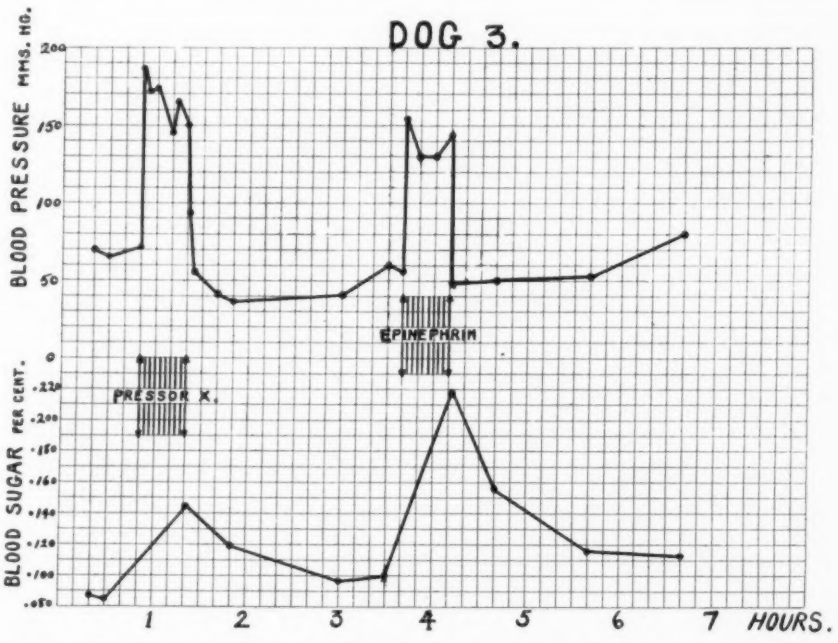


Fig. 7.—Continuous intravenous injections of 3 c.c. pressor X tests (1) in 150 c.c. saline, and 1.5 c.c. 1/1000 adrenalin chloride (Parke Davis) in 75 c.c. saline were administered over a period of half an hour, as indicated.

of similar doses (as judged by the pressor effect) of 1/1000 adrenalin chloride (Parke Davis), the influence of the pressor extracts on the blood sugar was relatively insignificant. (Fig. 6.) Injected intravenously into dogs, the pressor substance had a somewhat greater influence on the blood-sugar level. This effect, however, was still very much less than that produced by doses of epinephrin causing a rise in blood pressure of the same magnitude. (Fig. 7.)

Since the active principle responsible for this effect has not been isolated, the only method available for the approximate determination of dosage was comparison of the extent of the rise in blood pressure produced by injecting, into animals, comparable quantities of the two substances. Before further

work can be done, it will be necessary to evolve more accurate methods of assay, but since the problem cannot be continued with at the present time, it seemed to us important that the above facts be placed on record.

132 NASSAU STREET.

THE INFLUENCE OF P_H ON THE SELECTIVE BACTERIOSTATIC ACTION OF GENTIAN VIOLET ON MEMBERS OF THE COLON GROUP OF ORGANISMS*

BY W. D. STOVALL, M.D., M. STARR NICHOLS, PH.D., AND VERA VINCENT, B.A.,
MADISON, WIS.

IN A PREVIOUS paper† on the use of gentian violet in water analysis, we reported a marked difference in the toxicity of gentian violet for certain strains of B. coli. Winslow and Doloff published a similar report, 1922, which indicated a somewhat lower toxicity for certain of these strains than we found for our strains. Hall and Ellefson reported the satisfactory use of this dye in much lower dilutions in milk and water examinations. In the previous work for obvious reasons we kept the reaction of the media constant, varied the strength of the dye, and added the dye to the media in which the organisms were incubated. The bacteriostatic action of basic substances, dyes as well as other compounds and some of the factors which influence this action has been reported by Klinger, Davis, Norton and Davis, Churchman, Smith, Kunnwerde and Pratt, Beckwith, 1921; Browning, Gulbransen and Henneway, 1920, and others. The work of Stearns and Stearns, 1924, on the "Chemical Mechanism of Bacterial Behavior with Special Reference to Bacteriostasis and Gram's Reaction" has offered chemical explanation of this phenomena. They presented data to show that the behavior of bacteria toward a dye is largely determined by the nature of the bacterial protein, this being an ampholyte combining with acid dyes on the acid side of their isoelectric point, and with basic dyes on the alkaline side. In our work we found that two closely related gram-negative organisms exhibited a marked difference in their sensitiveness to the bacteriostatic effect of a basic dye. Stearns and Stearns again in 1924 sighted still more data which tend to show that the phenomena of bacteriostasis is a purely chemical one. Besides the chemical composition of the dye they point out the importance of (1) composition of the media, (2) mass effect of bacteria, and (3) reaction of the media. Basic substances in the media would presumably aid the basic dye and increase its bacteriostatic effect and acid substances decrease it.

In our previous work we added the dye directly to the media. We, therefore, decided to set up a series of experiments in which the dye and

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*From Wisconsin State Laboratory of Hygiene, University of Wisconsin, Madison, Wisconsin.

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†See this Journal.

bacteria would be brought together outside of the media in which they were to be grown, and that we would vary the hydrogen-ion concentration of the mixtures (dye and bacterial suspension), and the length of time of exposure of the bacteria to the dye. We hoped in this way we might be able to demonstrate the effect, if any, of the P_H on the sensitiveness of these two strains of different groups of B. coli to gentian violet and to exclude all factors influencing this difference except the character of the bacterial protein.

EXPERIMENTAL WORK

We experienced some difficulty in selecting a suitable buffer mixture that would permit a homogenous solution of the dye and would not effect the organisms. Clark's (1917) buffer solutions were first tried, but owing to the fact that these salt solutions invariably precipitated much of the dye, and the inhibiting effect of the salts were an unknown quantity; they were abandoned. For similar reasons Sorenson's (1912) buffer solutions were not employed. Witte's peptone was finally decided upon. It was found that a 1 per cent solution of this peptone could be easily adjusted to any desired P_H value by the addition of N/10 HCL of N/10 NaOH. It was also found that the gentian violet appeared to remain in true solution in this buffer mixture at P_H values of 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0, the values used in this work.

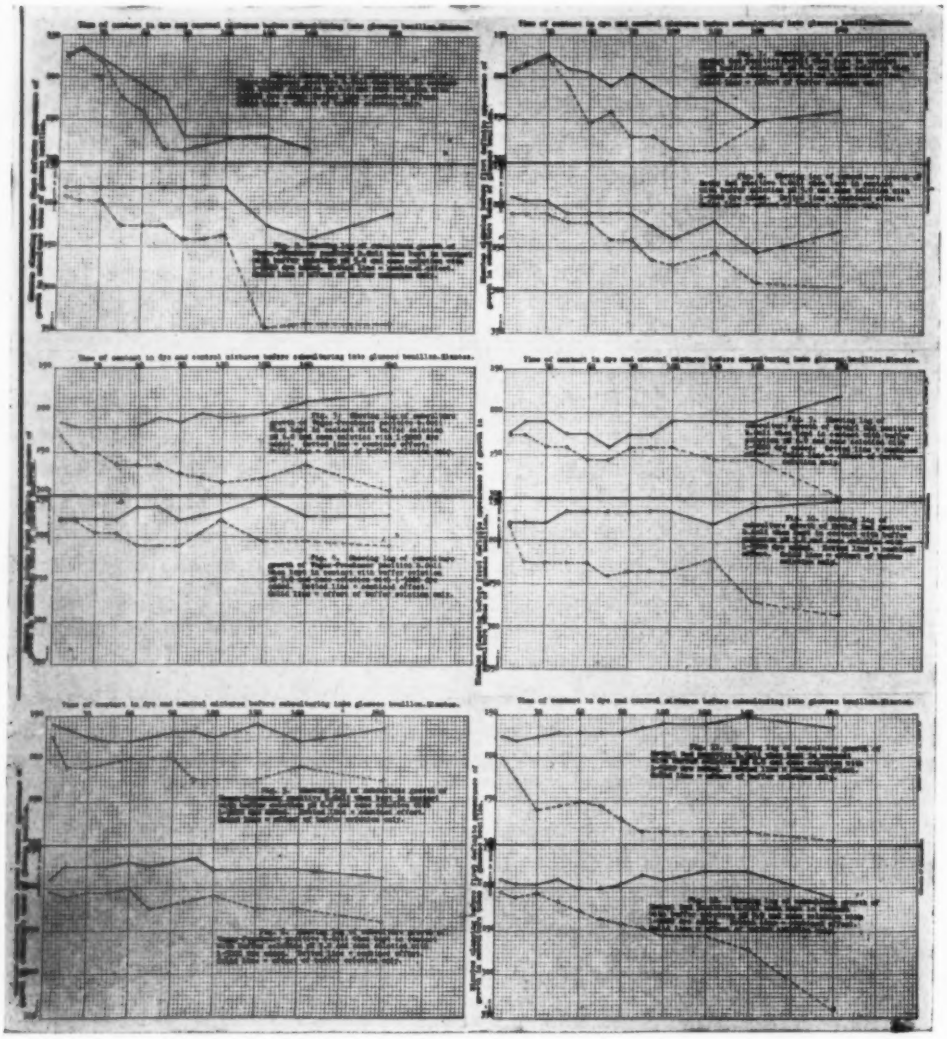
The peptone solution was made by dissolving 10 grams of Witte's peptone in 1000 c.c. of distilled water. This solution was boiled and filtered. The adjustment to the desired P_H value was made by addition of N/10 HCL or N/10 NaOH and comparison with standard buffer solutions. The indicators used were Clark's series except for the P_H value 4.0 and in this case Methyl orange (La Motte) was substituted for Brom phenol blue. These buffer solutions were adjusted, boiled, filtered, and given a final check before use in the experiments.

The dye concentration of 1-5000 (National Aniline Chemical, Chemical Co. "Gentian Violet 6B" lot number 2033) was used throughout these experiments. This strength was used because we desired to get a definite inhibitory action.

The exact procedure for exposing the organisms to the action of the dye buffer mixtures was as follows:

"Methyl Red positive organisms strain I and Voges-Proskauer positive organisms strain 18 of our previous work (1926) were the organisms used. The suspensions of organisms were obtained by washing a twenty-four-hour growth from an agar slant with 2 c.c. of sterile salt solution. From this suspension 1 c.c. was pipetted to 15 c.c. of the buffer mixture of the desired P_H value. This constituted the standard suspension for the experiment. To 40 c.c. of the buffer solution of desired P_H value was added 1 c.c. of 1 per cent aqueous solution of the gentian violet and 10 c.c. of the standard suspension. This is the dye buffer contact mixture. For the buffer contact mixture without dye hereafter known as "control," 8 c.c. of the desired buffer mixture was mixed with 2 c.c. of above standard suspension. The temperature of the mixture during the time of contact with bacteria was approximately 22° C.

At the end of definite periods of time after the preparation of these contact mixtures, viz., 5, 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, and 240 minutes, subcultures were made into glucose bouillon by withdrawing 0.1 c.c. from the contact dye mixtures and the controls. These subcultures were incubated at body temperature and observed for the first slight but definite

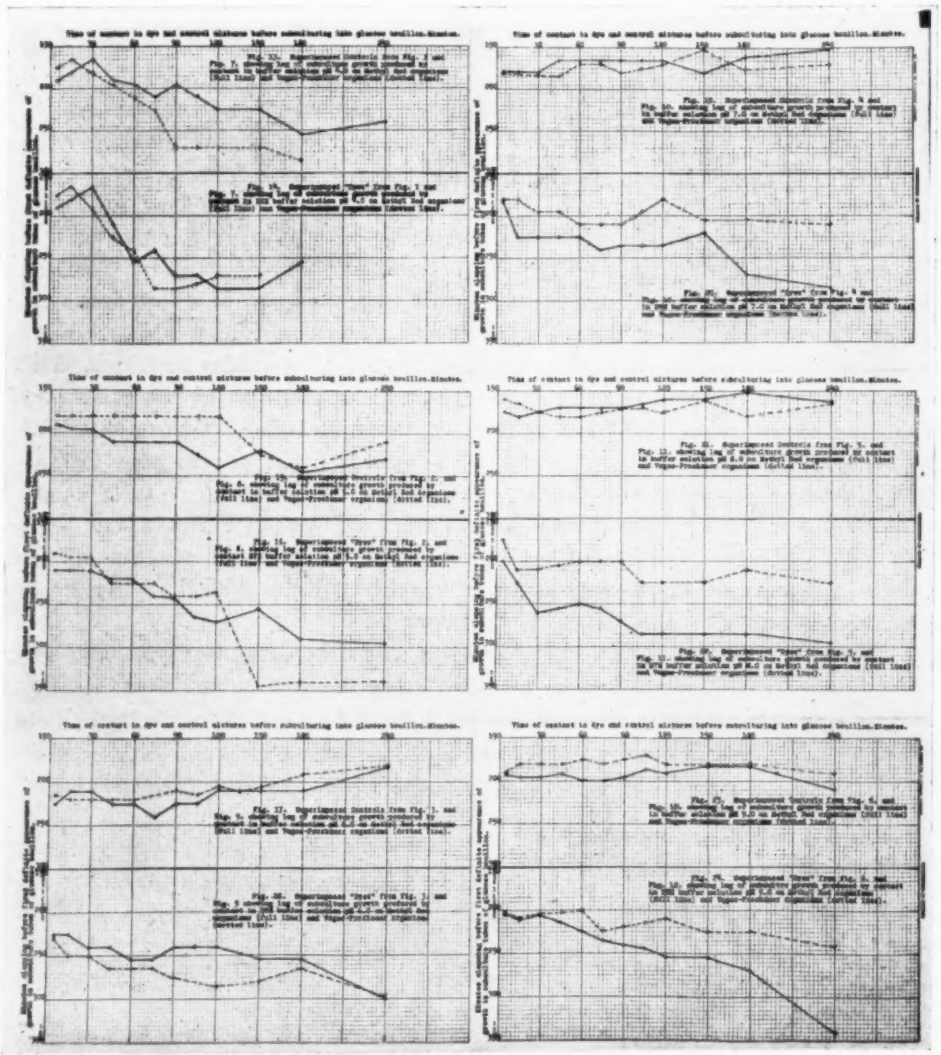


Figs. 1-12.

appearance of turbidity. This method of showing the lag of subculture growth is accurate to within ten minutes. The results of these experiments are graphically shown in the charts, Figs. 1 to 12, inclusive. Comparison of the effect of the dye and control mixtures on the growth of the two types of organisms are shown in Figs. 13 to 24, inclusive.

DISCUSSION

Comparison of Figs. 1 to 6 show that the greatest effect of the dye is evidenced at P_H 5.0 and 6.0 for Voges-Proskauer organisms while at P_H 4.0 the dye effect is closely approached by the buffer solution without the dye.



Figs. 13-24.

It is seen that at P_H 6.0 the dye has its greatest effect upon Voges-Proskauer organisms for at this P_H the buffer solution is shown to have practically no effect.

Comparison of Figs. 7 to 12, inclusive, show that the greatest inhibitive effect of the dye is evidenced at P_H 7.0 and 8.0 for the Methyl Red organisms. The strongest inhibition is shown in Fig. 11 at P_H 8.0.

For both types of organisms it is seen that the buffer showed no effect on subculture growth at P_H values of 6.0, 7.0, 8.0, and 9.0.

In Figs. 13 to 24, inclusive, is shown the first twelve curves superimposed in their respective order to show the effect of buffer and of dye buffer on the two types of organisms, at each P_H value. Here again it is seen that the effect of the dye on Voges-Proskauer organisms is greatest at P_H 5.0 and 6.0 and greatest on the Methyl Red organisms at P_H 7.0 and 8.0. In the buffer solution alone the organisms behave quite alike except that the aversion of the Methyl Red type to alkali and the Voges-Proskauer type to acid is evidenced to a slight degree.

SUMMARY

Using buffered solutions which at P_H values above 6.0 showed no inhibitive action on the growth of the organisms, it was possible to demonstrate the influence of P_H on the selective bacteriostatic effect of gentian violet on certain strains of B. coli. By these experiments we have eliminated the interfering action of the products of bacterial growth in media when the dye is added to the media. It is also apparent that the P_H values to which these organisms were exposed to the dye determined the strength of the inhibitory action of the dye, and that the growth of two members of the colon group of organisms (methyl red positive and Voges-Proskauer positive) are affected greatest by the dye at different degrees of acidity. Following Stearn's explanation of the bacteriostatic action of dyes our results indicate that there is a wide difference in the isoelectric range of the bacterial protein of two closely related strains of gram-negative organisms, and that the gram-negative reaction covers a wide range of P_H values.

These results also indicate that gentian violet has its most selective bacteriostatic effect on members of the colon group when acting in a solution buffered to a P_H of 8.0, and the action is markedly selective in this group between P_H 7.0 and 8.0.

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THE OCCURRENCE OF PARATYPHOID AGGLUTININS IN SERA TESTED FOR TYPHOID AGGLUTINATION*

By Robert A. Kilduffe, A.M., M.D., Atlantic City, N. J.

PRACTICALLY all serologists are agreed upon the fact that the macroscopic method of conducting the agglutination test for the presence of typhoid agglutinins is the method of choice and, whenever the character of the specimen permits this technic, it is customary to test for the presence of paratyphoid agglutinins as well.

Whenever paratyphoid as well as typhoid agglutination occurs, there are two possibilities: (a) the paratyphoid agglutination may be an instance of group agglutination; or, (b) the paratyphoid agglutination may be evidence of paratyphoid fever. The differentiation between these two possibilities is achieved, of course, by recording changes in the agglutinin titer upon a repetition of the test or by absorption tests.

Paratyphoid fever is an infrequent disease and the presence of paratyphoid agglutinins is commonly regarded as a relatively infrequent occurrence in the routine typhoid agglutination test so that, at times, it becomes a matter for decision as to whether it is worth while to conduct all three tests on sera submitted for routine examination.

A survey of this phase of the question has recently been reported by Gilbert and Groesbeck.[1] Of 13,644 sera routinely tested, an average of 0.09 per cent showed complete agglutination of paratyphoid bacilli and 0.7 per cent showed partial agglutination.

As a result of these findings these observers concluded that the test for paratyphoid agglutinins could be omitted from the routine examination except when paratyphoid infection was suspected.

While the clinical course and treatment of typhoid and paratyphoid infections have much in common, their differentiation is desirable in the interests of exact diagnosis and for statistical purposes, and, especially as paratyphoid fever may be overlooked or unsuspected without suggestive leads from the agglutination reaction, it has always been the custom in these laboratories to conduct the three tests simultaneously on each serum examined.

The macroscopic technic using formalinized suspensions, serum dilutions of 1:20 to 1:640, with adequate controls is used. In view of the conclusions of Gilbert and Groesbeck it was thought worth while to survey all the tests made in these laboratories from 1925 to 1927, inclusive, a total of 495.

Of these 188 or 37.9 per cent agglutinated B. typhosus, the average dilution of the serum in which agglutination occurred being 1:187.

*From the Laboratories of The Atlantic City Hospital.
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[1]Gilbert R. and Groesbeck, W. M.: The Occurrence of Paratyphoid Agglutinins In Specimens Submitted For The Typhoid Agglutination Test. Jour. Lab. and Clin. Med., 1925, x, No. 4, 282.

B. paratyphosus A was agglutinated by 54 sera or 10.9 per cent, the average titer being 1:156, while B. paratyphosus B was agglutinated by 75 sera, or 15.1 per cent, the average agglutination titer being 1:269.

Paratyphoid agglutination was encountered, therefore, in 129 sera or 27 per cent.

All of the positive cases in which clinical disease was present were bacteriologically proved to be typhoid fever and a case of paratyphoid infection was not encountered. As all the paratyphoid agglutinations were group agglutinations, therefore, the strength of the reactions is of some interest.

The lowest agglutination possible within the limits of the test was 1:20 and the highest 1:640.

The varying agglutinin titers for paratyphoid bacilli of the 129 sera in which paratyphoid agglutination occurred are shown in Tables I and II.

TABLE I

PARATYPHOID A AGGLUTINATIONS

SERUM DILUTION	NO. POSITIVE SERA	PER CENT
1:20	3	2.3
1:40	6	4.6
1:80	19	16.0
1:160	17	15.0
1:320	4	3.1
1:640	4	3.1

TABLE II

PARATYPHOID B AGGLUTINATIONS

SERUM DILUTION	NO. POSITIVE SERA	PER CENT
1:20	2	1.5
1:40	8	6.1
1:80	14	1.0
1:160	18	13.9
1:320	16	12.0
1:640	17	13.0

The relatively high agglutinin titer of these group agglutinations encountered with an unexpected frequency would have led to a suspicion of paratyphoid infection or its presence as a mixed infection were it not for the fact that the clinical course of the disease, the typhoid agglutinin titer, and the bacteriologic examinations of the blood, urine, and feces sufficed to prove the diagnosis of typhoid fever.

In this series, small though it be, the findings of Gilbert and Groesbeck as concerns the infrequency of paratyphoid agglutinins are not confirmed, possibly because the macroscopic technic which is somewhat more delicate, was used as compared to the microscopic method used in their series.

For the present, at least, it will be the custom of these laboratories to test each serum against all strains.

EVIDENCE OF THE SPECIFICITY OF THE INTRACUTANEOUS POLLEN TEST IN MAN*

By R. W. Lamson, Ph.D., M.D., and Gordon Alles, Ph.D., Los Angeles

THE intracutaneous test with pollen extract is frequently used as an aid to diagnosis in cases of asthma, hay fever, or other clinical allergy. It is not employed by certain workers because it is assumed to be nonspecific, or at least to fail to distinguish sensitizations to closely related pollens. Several published reports form the basis for such beliefs. Cooke[1] and his associates used this test in a study of grass pollen sensitizations and concluded that "an individual reacting to one reacts to all, which bespeaks a biological identity of the proteins derived from the pollens of the graminaceae." Coca[2] found a single exception to this rule. Brown[3] agreed with Cooke and extended the previous conclusions in that he believes "that patients reacting to the pollen of short ragweed will usually react also to other members of the compositae." VanderVeer[4] and Kahn[5] apparently agree with the previous views. Others have published observations pointing to an identity of the excitants in low and high ragweed. Walker[6] in a small series obtained little evidence of identity of any of the so-called fall pollens. He reports but one case that reacted equally strongly to all members of the compositae. In view of these theories it seems logical to speculate as to whether these observations are but manifestations of a fundamental biologic law governing all groups of hay fever plants. The observations which follow are submitted as evidence in this connection.

Two important components of the test must be taken into account, though each cannot be studied entirely separate. First, the reactive capacity of the skin may be assumed to be incapable of demonstrating specificity. That is, any protein solution or irritant might, when injected into the skin, produce an urticaria-like response, or the material tested might react one day and not the next and such alternation continue without end. If this were the case, of course, no significance could be attached to the test. The second factor is concerned with the reactive constituents in the pollen extract. If these are identical within a family or genus, then a test with a single pollen should indicate skin sensitization to pollens of that particular group. Chemical determination of nitrogen partition or attempts to establish any particular chemical constituent as characteristic of a single pollen do not, as yet, yield results that may be identified with species or other botanic subdivision. A recent study[7] in this laboratory of the micro-morphologic characteristics of grass pollens disclosed no significant difference which could be correlated with genera, though certain differences were noted among the fall pollens.

*From the Allergy Clinic of Dr. George Piness, Los Angeles, California.

The present study was designed to furnish data relative to the problems indicated above. The first point considered was duplication of the tests; the second, skin reactions to several species within a genus and in one case to two subspecies within a species. Some of the data have been indicated in a preliminary report,[8] and we now wish to present the complete data.

METHODS

The method of making pollen extracts has been outlined in detail in another article.[9] The test solution is a 1-1000 extract. Approximately 0.02 c.c. of this solution is injected into the skin on the lateral aspect of the upper arm. The reactions are read in fifteen to twenty minutes. The method of recording results is based on a numerical estimate of the degree of the reaction. A four-plus reaction is the largest and has definite pseudopods. It would correspond to McLaughlin's[10] Fig. 1. His Fig. 2 we would call a three-plus; the wheal is enlarged but not to the extent seen in the more positive reaction. One or more small pseudopods must be present. All other reactions which show differences from the control are considered doubtful. They are recorded as two-plus, but most of them are undoubtedly negative. The tendency is to overestimate the degree of positiveness. We lean to the side of conservatism but the borderline reaction is always difficult to evaluate. A marked two-plus reaction might actually be a weak three-plus, etc. Sex and age distribution are quite uniform throughout the several groups. These patients are known to have a clinical allergy, asthma and hay fever being the most common diagnoses in the series.

One of the most important criteria in judging any analytical method is the frequency with which duplication of results may be obtained. We have applied this measure to the intracutaneous test and the results obtained are given in Table I.

The extracts of four different pollens were employed in this series of 27 allergic patients. Each pollen was tested twice using solution from two separate bottles, A and B. The tests were placed about one inch apart in the long axis of the arm. The first 10 cases are those giving definitely positive reactions to Cynodon dactylon in duplicate and to practically no other pollen. In the last four of these, moderately positive three-plus reactions were obtained to some of the other pollens on one of the duplicates and in most cases the second test was recorded as a two-plus. Cases 11 through 15 gave definite reactions to Ambrosia psilostachya in duplicate, and in the last case only was there a mild reaction to another pollen, and the second test of this pair was considered doubtful. Probably these discrepancies in duplicates are instances of borderline reactions. A study of the data presented will indicate exact duplication of the reactions both positive and negative in the same patient in the majority of the cases. Other studies[9, 11] have shown that duplication of a majority of skin reactions to pollen is possible even when a two-week interval elapsed between tests. Absolute mathematical precision is not claimed for this skin test, but it does appear to have an accuracy comparable to other tests involving biologic factors.

TABLE I

DUPLICATION OF INTRACUTANEOUS TESTS

CASE NO.	AMBROSIA PSILOSTACHYA		ARTEMISIA TRIDENTATA		ATRIPLEX POLYCARPA		CYNODON DACTYLON	
	A	B	A	B	A	B	A	B
1	–	–	–	–	–	–	4	4
2	2	–	–	–	–	–	4	4
3	2	2	–	–	–	–	4	4
4	–	–	–	–	2	–	4	4
5	2	2	–	–	–	–	4	4
6	2	2	2	2	–	–	3	3
7	–	2	–	–	3	–	4	4
8	–	–	–	–	2	3	3	3
9	3	2	3	2	–	3	4	4
10	–	2	3	–	2	–	4	4
11	4	3	–	–	–	–	–	–
12	4	4	–	–	–	–	–	–
13	3	3	–	–	–	–	2	–
14	4	4	–	–	2	–	–	–
15	4	4	2	–	3	2	–	–
16	4	4	4	4	–	–	–	–
17	4	3	4	4	–	–	–	–
18	3	3	–	–	–	–	4	4
19	–	–	4	4	–	–	4	4
20	–	–	4	4	–	–	3	3
21	4	3	2	2	4	4	4	4
22	3	3	4	4	4	3	2	–
23	4	4	4	4	4	4	4	3
24	4	4	4	4	4	4	4	4
25	4	4	4	4	4	4	?	?
26	4	4	4	4	?	?	4	4
27	4	4	3	3	?	?	3	3

In Table II a summary is presented of the reactions obtained on 105 patients with pollens from four different genera. The pollen of ragweed reacted in 75 per cent of the cases and Allscale pollen in about 43 per cent. The pollen of Bermuda grass, a common sensitization in this section of the country, reacted in about 50 per cent of the cases.

TABLE II

DISTRIBUTION OF REACTION TO FOUR COMMON FALL POLLENS. TOTAL NUMBER OF PATIENTS OBSERVED, 105; 61 MALES AND 44 FEMALES

POLLEN TESTED	NO. REACTING
Ambrosia psilostachya (Western ragweed)	75 patients
Artemisia tridentata (Sagebrush)	51 "
Atriplex polycarpa (Allscale)	44 "
Cynodon dactylon (Bermuda grass)	55 "

These two tables furnish data tending to refute the claim that an individual skin sensitive to one of the compositae is usually sensitive to other members of this family or that there is necessarily any correlation between the reactive substance in pollens more widely separated than those genera within the compositae.

In Tables III, IV, and V we will attempt to show that the skin reactions to pollens within a genus are not uniformly positive or uniformly negative on the same patient. The individuals considered in Tables III and IV were tested with six species of Artemisia, one of these being represented by two subspecies. Twenty-two per cent of those tested reacted to but one of the six pollens, and 60 per cent of them reacted to not more than three (one-half) of the pollens tested. In the subspecies of Artemisia vulgaris the botanic differences are but minor ones, yet 24 out of the 50 patients tested reacted to but one of the two subspecies. It will be noted that no pollen consistently gave negative reactions, and that none reacted on all the patients tested. Although the reactive factor was shown to be present in each extract this factor was inactive in the absence of skin sensitization.

In Table V essentially similar results are shown for the seven species of Atriplex studied. Over 20 per cent of the patients reacted to but one pollen and 65 per cent reacted to not more than three pollens of the seven.

TABLE III

DISTRIBUTION OF REACTION AMONG SIX SPECIES OF ARTEMISIA (SAGEBRUSH)

		SPECIES					
NO. OF POLLENS REACTED TO	NO. OF PATIENTS	BIENNIS	CALIFORNICA	CAMPESTRIS	DRACUNCULUS	TRIDENTATA	VULGARIS[2]
1	18	4	4	3	1	4	2
2	19	6	2	3	7	10	11
3	12	6	4	7	6	7	6
4	9	5	5	6	5	7	8
5	8	8	5	7	6	7	7
6	16	16	16	16	16	16	16
—	—	—	—	—	—	—	—
Totals	82	45	36	42	41	51	50

TABLE IV

DISTRIBUTION OF REACTIONS AMONG SUBSPECIES OF ARTEMISIA VULGARIS—SP. (MUGWORT)

NO. OF PATIENTS	REACTING TO BOTH	REACTING TO LUDOVICIANA ONLY	REACTING TO HETEROPHYLLA ONLY
50	26	18	6

The data in these tables indicate that an allergic patient does not necessarily react to all "fall" pollens if he reacts to one; he may react to but one within a genus or to but one of a subspecies. The reaction, therefore, seems capable of differentiating between pollens even though chemical criteria are inadequate. This phenomenon has been demonstrated in a study of reactions to grass pollens[12] wherein it was shown that a definite skin hypersensitive individual might consistently remain negative to one or more common grass pollens, in spite of injection of these pollens and natural exposure to them for one or more years.

In the preceding discussions we have purposely avoided any reference to the clinical significance of the particular skin reactions. Nothing in the data presented could be construed as evidence in favor of or against a direct relationship between the skin reactions and the clinical significance thereof. These

data are presented and analyzed from the standpoint of the skin reactions only and seem to justify us in making certain deductions.

TABLE V

DISTRIBUTION OF REACTIONS AMONG SEVEN SPECIES OF ATRIPLEX (SCALES)

NO. OF POLLENS REACTED TO	NO. OF PATIENTS	SPECIES						
		ARGENTEA	BRACTEOSA	CANESCENS	LENTIFORMIS	PATULA	POLYCARPA	ROSEA
1	16	4	2	2	3	1	2	2
2	14	1	6	5	5	2	2	7
3	17	10	10	8	5	8	5	5
4	9	5	4	6	3	5	8	5
5	8	2	7	7	3	6	8	7
6	3	3	3	2	3	2	2	3
7	5	5	5	5	5	5	5	5
Totals	72	30	37	35	27	29	32	34

CONCLUSIONS

1. By the employment of the methods outlined, duplication of intracutaneous tests with pollen extracts in the skin hypersensitive individual was possible in a large majority of the cases.

2. A strongly positive skin reaction to one pollen in the allergic patient is not necessarily accompanied by a corresponding skin sensitivity to all other pollens. In fact the majority of patients did not react positively to one-half of the pollens within a genus. Evidence is submitted to show that a similar phenomenon may be manifested when pollens within a subspecies are tested.

3. These findings are considered evidence that the intracutaneous test is specific; and *a priori* that the reactive capacity of the patient's skin, and the reacting substance in the pollen extract manifest specificity. The test may be of value in separating closely related pollens even though morphologic studies of the pollen grains or chemical examination of the extract are inconclusive.

4. Absolutely no attempt is made to correlate these reactions with the etiology in an allergic patient.

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HEMOAGGLUTINATION

1. Hemoagglutination in the Blood of Infants

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BY THIS term is understood that peculiar phenomenon known as the clumping of erythrocytes when mixed with the serum of another animal. If the animals belong to the same general species, it is known as iso-agglutination; if they be of different species, it is called hetero-agglutination.

For many years it has been known that blood, withdrawn from one animal and injected into the blood stream of another, will often produce immediate, alarming, and even fatal results. During the past quarter of a century much scientific inquiry has been made to determine the cause and nature of this reaction. Landsteiner, in 1901, found after a study of the blood in a small series of cases, that the fatal results were probably due to agglutination of the red blood cells; he also found that human blood could be divided into three distinct groups. Heektoen in 1907 confirmed these findings, and Jansky in the same year added another group, making four in all, each marked by characteristic reactions. Accordingly, he worked out a table for the classification of human blood based on the phenomenon of iso-agglutination. Some two or three years later, Moss, working independently, framed a table identical with the Jansky classification, except that Groups I and IV are in reverse order. For some reason, other than right of discovery, the Moss system has been most generally adopted. It is the one used in this paper, although full credit belongs to Jansky because of his prior work.

Since 1910 many observers have invaded this interesting serologic field, and separately they have arrived at conclusions more or less contradictory. It is not the intention to contrast those findings or pass upon the claims of the various investigators, except as their reports deal with problems that lie within or adjacent to the province of this paper, namely, the blood of infants.

During the late World War when transfusion became so urgent because of the numerous cases of hemorrhage, quick, easy methods of blood typing were highly desirable, and several simple procedures were evolved. In general, they all fall under two groups, the macroscopic and microscopic. The former method involves the use of serologic tubes carrying mixtures of red

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cells and serum, together with controls. The agglutination is seen as masses of clumped red cells which sink to the bottom of the tubes. While this method is accurate and highly satisfactory in the hands of trained experts, it offers no advantage over the quick and simple microscopic method for the average typist. The latter method, therefore, has been followed exclusively.

Technic employed:

1. *Moss' Classification.*—

SERA TYPES

CELL GROUPS	1	2	3	4	PER CENT
I	0	+	+	+	8
II	0	0	+	+	40
III	0	+	0	+	10
IV	0	0	0	0	42

Explanation of Table:

Group I. The serum of Type I does not agglutinate the red cells of any of the four groups of cells; the corpuscles of that group, however, are clumped by the sera of the other three types.

Group II. Type II gives a positive reaction with Groups I and III; negative with II and IV.

Group III. The Type III serum will agglutinate the red cells of Groups I and II; it will not clump those of Groups III and IV.

Group IV. While none of the four types of sera will agglutinate Group IV red cells its own serum will clump those of the three other groups.

2. *Vincent's Open Slide Agglutination Method.*—This method was followed exclusively because of its accuracy and simplicity. It consists of the following procedure:

T.2.S.	T.3.S.
0	0
R.C.X	R.C.X

a. Place one drop of No. 2 serum on the left end of the slide. Place one drop of No. 3 serum on the right end of the slide.

b. Mix one small loopful of prepared red cell suspension with each drop of serum, respectively, taking care to flame the loop before each mixing.

c. Red cell suspension is prepared by centrifuging the citrated blood, decanting off the supernatant fluid, then washing the cells in normal salt solution. Since the umbilical blood was collected in sterile normal salt solution containing 1 per cent sodium citrate, this portion of the procedure was found quite unnecessary; accordingly, loopsfull of red cells were taken directly from the vials. From making many tests, no difference could be detected in changed reactions or in the amount of time required for agglutination; therefore, this portion of the technic was omitted.

d. At regular intervals of two or three minutes, the slide should be agitated by alternate tilting to aid diffusion and hasten the process of clump-

ing. This is usually complete within ten or fifteen minutes. Cover-slips are not necessary if examined at once. The microscope should be used in every case.

3. *Stock Sera.*—Inactivated stock sera, Types II and III, adult, were secured from the Virginia Mason Laboratory and preserved in sealed, capillary tubes in the ice box. No deterioration in potency was detected before each quantity of stock sera was replenished, some two months after withdrawal. No preservative was used, and no special preparation required other than above mentioned.

4. *Umbilical Blood.*—This was obtained from various private hospitals through cooperation of obstetric departments and the attendant physicians. After delivery the umbilical cord was cut and drained into twin-batteries of two-dram vials, each, before expression. One empty vial received blood which was allowed to clot and the serum later removed as needed; the other vial, partly filled with 1 per cent sodium citrate in normal salt solution, received blood which did not clot. These specimens were preserved in an electric refrigerator and examined as soon as possible, usually within forty-eight hours after collection. Frequently, a week or so after the discovery of a good, strong reacting umbilical serum, its potency on retest with both the original and new red cells was well preserved. So far as determined, if both the red cells and serum were stored in the refrigerator and preserved from bacterial invasion, agglutination persisted. If the specimen was grossly contaminated, the clumping phenomenon was soon destroyed.

It should be noted here that the specimens of umbilical blood were secured under the most favorable conditions. The vials containing the citrate solution were twice autoclaved, once when prepared and again before going to the obstetric wards, to prevent contamination of the delivery room. The blood from the cord was then drawn into these sterile containers and, if not later contaminated, was preserved for a considerable period of time.

TABLE I

RESULTS OF TYPING UMBILICAL RED CELLS

TYPE	SEX	CASES	TOTAL CASES	TOTAL PER CENT
I	M	2		
	F	4	6	7.1/2
II	M	15		
	F	17	32	40
III	M	3		
	F	5	8	10
IV	M	18		
	F	16	34	42.1/2
Total	M	38		
	F	42	80	100

Explanation of Table I.—As stated before, the red cells were tested with fresh human serum of known types, II and III, for group determination. It will be seen that the percentages found for the four different general types

of umbilical blood correspond quite closely with the percentages usually alloted for adult blood. In other words, the agglutinogenic property of erythrocytes appears fully developed and quite normal at birth. There existed no opportunity to recheck the infants blood at a later period to learn if the postpartum type persisted; neither was it possible to compare the fetal types with those of maternal blood.

Of the eighty cases examined, the sexes are represented about equally, thirty-eight males and forty-two females. Little difference could be found in this series of typings from that standpoint, except perhaps, a slightly higher percentage of the less common groups for girls.

TABLE II

RESULTS OF CROSS-AGGLUTINATING UMBILICAL CELLS WITH UMBILICAL SERUM

SERUM X. 0 R.C.Y.		SERUM Y. 0 R.C.X.
	NO. CASES	PER CENT
Both positive	8	11.2/3
One positive	24	35.1/3
Neither positive	36	53
Total cross-agg.	68	100

Explanation of Table II.—This shows a series of sixty-eight cases of cross-agglutinations, e.g., umbilical serum X, was mixed with umbilical red cells Y, while the blood serum of Y was typed with the red cells of X, respectively, on opposite ends of a microscopic slide, as shown in the table heading. Agglutination occurred in only eight cases, simultaneously, at both ends of the slide; in twenty-four cases a single test showed positive; while in thirty-six trials, neither mixture showed agglutination. It will be further noted that the typings were made in pairs, so that the sixty-eight cases represent, in reality, one hundred thirty-six trials. According to the Moss classification, Type I serum will agglutinate neither of the four groups of cells; nor are the cells of Group IV clumped by any of the normal types of sera. It follows, therefore, that combinations of sera and cells made at random will include many negative tests. Of the combinations tabulated above, i.e., the one hundred thirty-six trial agglutinations, seventy-one were theoretically positive but only forty showed positive by actual test. In other words, of the theoretically positive reactions only 56 per cent showed the clumping phenomenon. In some cases these reactions were vigorous and appeared promptly; others proved weak and somewhat delayed; still others, which should have occurred, did not appear. It is, therefore, quite apparent that the agglutinin of umbilical serum does not show the same degree of reactivity as shown by the serum of an adult.

In Tables I and II, the findings confirm in a general way the observations of De Biasi, reported in 1923. He found in a series of one hundred cases of umbilical blood, the following group percentages, according to the Moss' classification:

Group I	12 per cent
Group II	24 per cent
Group III	17 per cent
Group IV	47 per cent

He also found that it was possible to arrange the red cells according to this classification scheme. While his percentages differ slightly from these findings, as shown in Table I, nevertheless they are as a whole, confirmed. Therefore, his statement may be concurred in that "corpuscles of the newborn infants have their quota of receptors."

As regards his findings in infant serums, in no cases were the maternal red cells agglutinated by the serum of the infant; in four cases only did he find that the infants red cells were clumped by maternal serum. This result one would naturally anticipate, since the red cells of the child are developed from its own tissues; whereas there must necessarily exist some osmotic exchange between maternal and fetal sera, in other words, the serum of the newborn is a mixture. By cross-typing one umbilical blood with another, agglutinin if present and well developed in the serum of the newborn should give the same reactions and percentages shown by normal adult sera when typed against these cells. This is not true, as shown in Table II. In only about one-half the cases are theoretically positive agglutinations confirmed by laboratory tests.

CONCLUSIONS

I. The erythrocytes of umbilical blood, react in a normal way, and give the same group percentages shown by adults; their agglutinophyllic power, therefore, appears fully developed at time of birth.

II. Umbilical serum shows in a positive way, its theoretic reaction in about 50 per cent of cases; many of these when present are weak with action somewhat delayed. It appears, therefore, that its agglutinin is often immature or not fully developed at time of birth.

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LABORATORY METHODS

A BLOOD STAIN GIVING MORE CONSTANT RESULTS*

A New Departure in Staining With Romanowsky Stains Which Eliminates a Number of Sources of Error

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DIFFICULTIES WITH WRIGHT'S STAIN

MOST everyone has had difficulties at times getting satisfactory results with the present methods of staining with the various modifications of Romanowsky stains. Sometimes a specimen of Wright's stain will fail to stain properly and has to be discarded, whereas the next sample, made up apparently in the same way and from the same ingredients, will prove quite satisfactory.

At other times the difficulties may arise from failing to remember the staining time or the proper amount of water needed to dilute the stain, or from accidental bungling in handling the slide during the process of staining. As a result some smears are not stained deeply enough for use, while others are heavily stained and perhaps covered with a dense film of precipitate which obscures all details. The beginner particularly is prone to have difficulty in obtaining well stained smears.

Often these failures are discouraging because it is difficult to locate definitely the factor causing the poor results. On several occasions we have happened to have poor luck just when we had a set of particularly interesting slides which we wished to stain for class work or for demonstration purposes. We have also observed, in looking over loan collections in other departments and other schools, that blood smears are often so poorly stained that they fail to show the things which they are supposed to illustrate.

For these reasons we undertook some time ago to investigate the factors responsible for failures in getting proper results with Wright's stain, hoping at the same time to hit upon a method which would give more constant results.

SEARCH FOR A DECOLORIZING SOLUTION

Since overstaining and precipitated stain seemed so often to be the crux of the difficulty, it seemed to us that one way out would be to purposely overstain the smears and then partially decolorize them. We had already made use of the expedient at times of removing precipitate with ethyl alcohol. However, it was quite easy to completely decolorize the smear in this way, the nuclear blues being particularly attacked, so some slower acting and more differential decolorizing solution had to be found.

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Experimentation with all of the other common solvents used in the laboratory proved fruitless. They removed either all or none of the stain. Mixtures of these solvents in varying proportions likewise proved useless.

It occurred to us that something might be added to the ethyl alcohol to slow its action or make it more differential. Since the blues were especially attacked by this solvent, we added varying quantities of methylene blue, finding that a saturated solution gave fair results, but much of the azure was lost. This fact suggested the use of Wright's stain, itself, in the decolorizing solution, as the azures might then be retained. The results obtained with this solution, after learning to adjust the factors, as described below, which cause variations in the stains, were very satisfactory.

PREPARATION OF SOLUTIONS

Solution I.—This consists of Wright's stain dissolved in absolute methyl alcohol in the usual way. However, we prefer to prepare the solution by placing enough of the dry powder into a dry clean bottle to saturate the amount of alcohol added (not more than about 0.3 gm. will dissolve in 100 c.c.). The solution should be allowed to stand for several hours, preferably a day or two, with occasional shaking. It should then be filtered and diluted with one-fifth of its volume of methyl alcohol.

Solution II.—This consists of a saturated solution of Wright's stain in 85 per cent to 90 per cent ethyl alcohol (only about 0.2 gm. will dissolve in 100 c.c.). We usually prepare this solution by using nine parts of 95 per cent alcohol to one part of distilled water as the solvent. This solution must stand for not less than twenty-four hours, preferably longer, with occasional shaking before it can be used, because saturation is slowly reached when the excess of stain present is so small. It is well to filter before using, otherwise some of the undissolved stain is likely to be poured on the slide and fail to be washed off.

DIRECTIONS FOR USING STAIN

1. Drop on just sufficient stain (Solution I) to cover blood smear; *drain off excess stain* at once; let stand until all of the stain remaining on the slide *turns red.*

2. Flood with distilled water and allow to stand for one or two minutes.

3. Wash with decolorizing solution (Solution II) until most of the red precipitate disappears. That over the smear usually disappears immediately, and any at the ends of the slide may be wiped off with a cloth.

4. Wash with distilled water, dry and examine. *Note.* Drying at any stage *after the excess stain is drained off* does not materially harm the preparation.

FACTORS CAUSING VARIATIONS IN STAINING

During this study we have found at least four important factors which influence the character of colors obtained. Thus greater intensity of red is obtained by: (1) A more acid water; (2) a higher percentage alcohol in preparing Solution II; (3) a more concentrated Solution I; (4) a longer staining time in the presence of water. Conversely, a less intense red is produced

by the reverse of these factors. The four are to a certain extent interdependent, a change in one being compensated for by a change in another.

We have studied somewhat in detail the effect of the P_H upon the colors obtained. Using water buffered with phosphate solutions, we find that the colors which we prefer are obtained when the water is a P_H of 6.4 to 6.8. The colors are fairly satisfactory for differential counting with a P_H as low as 5.6, but the red cells are very pink and the nuclear blues of the leucocytes hazy and indistinct. On the other hand, when the P_H goes beyond 7.4 the red cells take on a greenish blue hue and the granules and nuclei of the leucocytes fail to take the eosin and azures properly.

Distilled water is usually slightly acid and consequently makes a satisfactory diluting and washing medium without buffering. However, the amount of acid is subject to variation, especially where the water is distilled from city supplies which are chemically treated. For this reason we prefer to use a water which is buffered.

A simple method of preparing such a water is to keep on hand 1 per cent solutions of KH_2PO_4 and Na_2HPO_4. Ten c.c. of the latter are used with each liter of distilled water, and the KH_2PO_4 is added in 1 to 5 c.c. quantities until the desired color of red cells is obtained when smears are stained. The quantity usually needed is 10 to 30 c.c., depending upon the personal preference as to shade. We like the color to be a slightly pinkish buff.

In regard to the second factor, the best strength of ethyl alcohol to use in preparing Solution II is 85 to 90 per cent. Stronger alcohol often fails to remove the red precipitate left in overstaining the smear, and weaker alcohols remove both reds and blues, even to complete decolorization.

As to the third factor, it often happens that a stain which sits in a dropping bottle for a long period loses alcohol by evaporation and becoming concentrated causes overstaining with eosin. Usually diluting again with methyl alcohol restores the proper balance of staining.

PIGMENTS USED WITH THIS PROCEDURE

Most of our experimentation has been done with Wright's stain produced by American manufacturers. All samples which we have tried have given satisfactory results. It seemed well, however, to discover if pigments made in other ways would be equally successful. For this reason we have prepared pigments by all of the processes usually employed in polychroming methylene blue and making the eosinates.

Several specimens have been prepared by Wright's, Leishman's, and Wilson's methods, and all were satisfactory. We have also procured samples of the latter, two from manufacturers, and tried them out, with equal success. Personally we like the qualities of the pigment produced by Wilson's method as described by Svehla[1] better than any of the others.

It is interesting to note that some of the samples used worked poorly when the usual staining method was employed, but gave beautiful stains with the method which we have devised.

ALCOHOLS USED IN PREPARING SOLUTIONS

In order to obtain good results with Wright's stain by the usual procedures it is generally advised to use a special absolutely pure, acetone free methyl alcohol. With this procedure we have been able to secure good results even with commercial wood alcohol, the principal disadvantages being that some samples contain impurities which are likely to leave more precipitate on the smear, and some are not near enough 99 per cent purity to properly fix the cells in the thick portions of the smear. However, this does not interfere materially with recognizing leucocytes or blood parasites, and for routine work, where the slides are discarded after examination, such alcohols are often satisfactory enough.

We have also found that the commercial denatured alcohols can be used in making Solution II, especially if wood alcohol is the principal material used in denaturing. Of course, there are a large number of formulas in use in denaturing, and no doubt some substances used in the process might render the sample unfit for this purpose.

We have procured samples of these two alcohols from a number of sources, drug stores, wholesale dealers, etc., and have used specimens on the shelves of various departmental laboratories, and none which have been refined enough to be perfectly clear and colorless have failed to give success.

CHARACTERISTICS OF STAINS OBTAINED

We have stained hundreds of slides by this procedure with very uniform results and practically no failures. It has worked satisfactorily in the hands of a number of students who have tried it out parallel with their Wright's stain. Likewise, a number of our personal friends in other medical schools have been kind enough to try out samples and have reported results comparable to our own.

The differentiation between the leucocytes is sharp. There is no massing of stain upon the leucocytes to mask internal structures, and the granules stand out plainly. The granules of the neutrophiles are quite delicate but discrete, those of the eosinophiles a definite red, and those of the basophiles a very deep blackish purple. Likewise, the azurophilic granules of the lymphocytes are very definitely demonstrated, even in smaller cells.

The red cells may be stained any color desired from a light yellowish buff to a very distinct pink, depending upon the proper adjustment of the factors mentioned above. Due to the delicate coloring of the erythrocytes, polychromatophilia and basophilic stippling are clearly demonstrated.

Fortunately, during the period of our experimenting, we have been able to obtain large numbers of smears from cases of leucemia, and of tertian and quartan malaria, and much of our staining has been measured by how satisfactorily the malarial parasites and the granules of the myelocytes were brought out.

SUMMARY

1. A method of overstaining with Wright's stain, and partially decolorizing, is described.

2. Factors causing variations and failures in staining, and their proper adjustment, are discussed.

3. Commercial alcohols can be used in routine work.

4. Constant satisfactory results are obtained by those who have used this method.

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RAPID AND ROUTINE PREPARATION OF TISSUE SECTIONS*

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DESPITE the fact that numerous technics for the rapid and routine preparation of tissue sections have been devised during the past ten years, many of the methods at present in use, while producing reasonably good routine sections, are too time consuming for the diagnosis of cases in which a radical surgical procedure is anticipated, should the tissue removed prove to be malignant. The freezing method with polychrome methylene blue staining answers the purpose extremely well when a definitely benign or malignant tissue is found, in as far as rapidity is concerned, but where a borderline tissue is to be pronounced definitely malicious or nonrecurrent, and a major operative procedure is to be undertaken or otherwise depending on the pathologist's interpretation of the sections, a grave feeling of responsibility and doubt of one's reading must necessarily ensue as he studies a section distorted by freezing and not showing the best detail possible as the result of poor polychrome stain infiltration.

The following method has been used by me for the past six years, and while consuming about six hours from the time the tissue is received until the time the surgeon has his report, the method is sufficiently fast for all practical purposes, at the same time producing permanent sections which it is a pleasure to interpret. The cells show no distortion or shrinkage, are free from artefacts, and show very definite clear cut nuclear and cytoplasmic stain detail. In addition to these facts the method is applicable to routine as well as urgent work. My laboratory has been in the habit of using the technic for all tissues, in this way cleaning up all the pathologic specimens the day after they are received as far as the surgeon is concerned, unless for some reason special staining methods are deemed advisable.

Pieces of tissue of sufficient area to facilitate an adequate study of the specimen are cut as thin as is convenient to handle and should in no case be over 2 mm. thick for the rapid method. The cutting surface may be of any size up to one by three inches, though three-quarters of an inch square or smaller has been found to be a convenient size for routine work. In case

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thicker pieces of tissue are desired, as for serial sections, they must necessarily remain longer in the fixing reagents and are not applicable to the rapid method. Also they are more subject to shrinkage from remaining in the fixatives longer. The tissues are threaded on a length of fine sewing cotton with the aid of a fine needle. A small glazed paper or parchment tag is threaded on, after all the sections from one source, bearing an indentification number in ink. In this way many sections or sets of sections may be placed on one thread. Uterine curettings are massed loosely and tied in a single layer of gauze before being placed on the thread.

Eight one-quart covered jars are required for the fixing reagents and two small enameled pitchers for the paraffin. Ordinary fruit jars with clamp tops are ideal for the reagents. The first jar is nearly filled with 85 per cent alcohol, the second with 90 per cent alcohol, and the third with 95 per cent alcohol. Acetone is placed in the fourth jar, oil of cedar leaves in the fifth, chloroform in the sixth and seventh, and a saturated solution of paraffin in chloroform in the eighth. The jars should be kept nearly full of reagent. One worker I saw using the technic was actually trying to get results with half an inch of oil of cedar leaves in the jar when he was running through six to eight tissues each day. As the alcohol becomes hydrated only the first needs to be discarded, the second replacing the first, the third the second, and only the third needing to be replaced by new. In time the first chloroform will become quite yellow from oil of cedar leaves and will need discarding. The other reagents will last almost indefinitely, needing only to have their volume replenished from time to time. This brings about an element of economy which is usually appreciated where large numbers of tissues are run through routinely. The two pitchers are numbered one and two and contain paraffin of about melting point 55° C., and are kept in the paraffin oven, which should be just hot enough to keep the paraffin liquid but should never be over 58° C. or the tissues will tend to scorch unless very closely watched.

The fresh threaded tissues are placed in the first alcohol for half an hour, transferred to the second for a similar period, and are then allowed to remain in the third for two hours. They are then run through the other reagents remaining fifteen minutes in each. No preliminary formalin or other fixation is necessary, the only advantage to be gained by a preliminary fixation being a tendency to distortion from shrinkage. Formalin or Zenker's fixed tissues run through this method stain equally as well as those not so treated. After fixation the tissues are placed in first one paraffin and then the other, remaining fifteen miutes in each.

While the tissues are in the oven, small paper boxes may be folded from any stiff paper large enough to accommodate one or more sections. These are filled with paraffin and the tissues unthreaded and immersed while the paraffin on them is still molten. The identification numbers are written on the ends of the boxes, and the boxes placed on the ice in the refrigerator to harden; a process which should take not more than twenty to thirty minutes at the most to obtain good hard blocks.

The paper is then removed and the blocks trimmed. Trimming is an important step if long ribbons of serial sections are desired. Two sides of the block should be trimmed fairly close to the tissue, and the other two, which will form the top and bottom edges when the block is in the microtome, should have a paraffin margin of approximately 3 mm. Good sections cannot be expected if the microtome knife is not sharp or has nicks in it.

Sections may be stained by any method desired, the following giving uniformly good results. After mounting of the tissues on glycerine albumin smeared slides they are set on edge in the paraffin oven until the paraffin has melted, usually five to ten minutes. The slides are then cooled either in the refrigerator or at room temperature just long enough for the paraffin to crystallize and are then run through the following stain process:

1. Xylol 1 for five minutes.

2. Wash by gently swishing back and forth in xylol 2 and 3, 95 per cent alcohol, and water for thirty seconds each.

3. Delafeld's hematoxylin fifteen minutes.

4. Rinse in water.

5. Decolorize in 1 per cent HCl in 70 per cent alcohol by dipping in slowly two or three times.

6. Wash in running water until blue; twenty minutes.

7. Counterstain in ½ per cent aqueous eosin three to five minutes.

8. Rinse in alcohols 1, 2, and 3.

9. Clear in 0.3 per cent carbolic acid in xylol three minutes.

10. Rinse in xylol 4 and 5 and leave in this until ready to mount.

11. Clean any undesired tissue off of the slide with a clean towel without letting the section become dry. Place a drop of xylol balsam on the center of the section and press a cover slip down on it.

This staining technic may be speeded up by leaving the slide in hematoxylin only five minutes, rinsing only momentarily in acid alcohol, and using very dilute ammonia water to develop the blue color instead of leaving in running water for twenty minutes.

In routine work the tissues may be kept in air tight jars until the afternoon, the gross examination then performed and the tissues run through as far as the third alcohol, remaining in this until the following morning.

Tissues cut and stained according to this technic should be free from artefacts, should cut and stain easily whether large or small, show good differentiation whether for microscopic study or photomicrographs, and should give as true a histologic picture as is at present possible to obtain.

A COMPARATIVE STUDY OF THE EFFICIENCY OF DEHYDRATED ENDO'S AGAR AND KRUMWIEDE'S TRIPLE-SUGAR AGAR*

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DEHYDRATED media of various kinds have been placed on the market by certain of the commercial laboratories. Such media, if they proved to be as efficient as media which had not been dehydrated, would be of great value in many small laboratories. These media which are prepared in the laboratory by dissolving the dry material in distilled water, dispensing, and sterilizing, would have the advantage not only of saving time and material but also of tending to make results obtained in one laboratory more comparable with those obtained in another through the use of more nearly uniform media.

This study was undertaken to compare the efficiency of dehydrated Endo's agar and Krumwiede's triple-sugar agar made by one of the commercial houses, with the corresponding standard media[1] used in this laboratory in the routine examination of specimens for organisms of the enteric-disease group. At the time this work was done, the standard Endo agar in routine use in this laboratory was made according to a modification of the formula of Robinson and Rettger,[2] and the triple-sugar medium was a modified[3] Russell's[4] double-sugar medium.

The efficiency of the two Endo agars was compared by determining: how well and typically strains of the colon-typhoid group of organisms grew on each of these media; and by the extent to which organisms found in fecal specimens containing incitants of enteric diseases could be differentiated from one another when grown on each of these agars. On the triple-sugar media, comparison was made of the reactions produced by pure cultures of various organisms. These media were further studied by observing the reactions produced in each by the growth of a single colony fished from Endo's agar inoculated with mixtures of B. coli and B. typhosus or B. dysenteriae, or with routine specimens containing organisms of the enteric-disease group.

TECHNIC

The cultures used were Staphylococcus aureus, B. coli, B. typhosus Bender strain, B. paratyphosus A, B. paratyphosus B, and three strains of B. dysenteriae, Flexner, Mt. Desert, and Shiga. The Endo-agar plates were inoculated with suspensions of these cultures made by emulsifying in 0.85 per cent salt solution the growth from agar slants which had been incubated for eighteen hours at 37° C. The tubes of triple-sugar media were inoculated directly from the eighteen-hour agar slant cultures.

The mixtures of B. typhosus and B. coli (1:5, 1:10, 1:15, 1:20) and of B. dysenteriae Mt. Desert and B. coli (1:6, 1:12, 1:24; 1:30, 1:36) were prepared from eighteen-hour broth cultures of the same density and diluted to very light suspensions with sterile broth.

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The specimens with which the Endo-agar media were tested were suspensions of feces in glycerin-salt solution.[5] All specimens used had already been found to contain B. typhosus, B. paratyphosus, or B. dysenteriae.

Several samples of both the dehydrated Endo agar and the dehydrated triple-sugar agar, were obtained from time to time and tested. The composition of two of the samples of dehydrated Endo's medium differed from a third sample both in kind and amount of the ingredients present and in the amount of dehydrated material required to make a liter of medium.

Sample 1		*Samples 2 and 3*	
Peptone	5.0 parts	Dipotassium phosphate	3.5 grams
Beef extract	3.0 parts	Peptone	10.0 grams
Lactose	10.0 parts	Lactose	10.0 grams
Agar	15.0 parts	Agar	15.0 grams
Basic fuchsin	0.1 part	Basic fuchsin	0.5 gram
Sodium sulphite q.s.		Sodium sulphite	2.5 grams
Dissolve 34 grams in one liter of distilled water		Dissolve 41.5 grams in one liter of distilled water	

The samples of dehydrated triple-sugar medium differed in composition only as to indicator. The last sample contained phenol red instead of Andrade's indicator.

Throughout the work with the Endo media, freshly poured plates of the medium made according to the modification of Robinson and Rettger's formula were used. The dehydrated medium was made exactly according to the directions on the container and inoculated and incubated on the same day. All tests were carried out in duplicate. Two plates of each medium were inoculated in the following way. One loopful of inoculum was spread over the agar surface of the first plate with a sterile glass rod which was then used to inoculate the second plate. The two plates of the second medium were inoculated in a similar manner. The plates were then incubated for eighteen hours at 37° C. This same procedure was followed with saline suspensions of pure cultures, with mixtures of cultures, and with feces.

The dehydrated triple-sugar medium was made, sterilized, and inoculated on the same day. Tubes of this medium and of the triple-sugar medium in routine use in this laboratory were inoculated with a straight needle both deep into the butt and on the slant surface. The incubation period was eighteen hours.

When mixtures of two cultures or feces containing organisms of the enteric-disease group were plated on the Endo media, colonies which had the characteristic appearance of typhoid or dysentery bacilli were fished to triple-sugar-agar media. A tube of each triple-sugar medium was inoculated from the same colony and these tubes were incubated together. Whenever the reaction produced in the dehydrated medium did not agree with that of the standard triple-sugar agar, other tubes of the latter were inoculated with the culture from the dehydrated triple-sugar medium. If these second tubes of routine medium did not agree with the first, the purity of the culture was investigated by means of Gram-stain preparations. One characteristic fishing from each of the Endo media inoculated with specimens of feces containing enteric-disease organisms was tested by macroscopic agglutination.

DISCUSSION

The uninoculated dehydrated Endo medium differed in appearance and in hydrogen-ion concentration from the Endo agar made according to the standard method. The latter medium was colorless and clear. The plates made from the sample of dehydrated medium which contained 0.1 part of basic fuchsin were clear and slightly colored by fuchsin. The plates made from the samples which contained 0.5 gram of basic fuchsin were deeper in

color and contained a heavy precipitate which was dispersed finely throughout when the medium was shaken just before pouring the plates. The hydrogen-ion concentrations of the dehydrated medium varied from P_H 7.4 to P_H 7.6 while that of the medium made in this laboratory was approximately P_H 7.8.

When plates made with the Endo media were inoculated with saline suspensions of B. coli, all plates developed large colonies with pink irregular edges and centers which varied in color from light red on the routine Endo agar and on the sample of dehydrated medium containing the smaller amount of basic fuchsin, to deep metallic red on plates of the samples of dehydrated medium which contained 0.5 gram of basic fuchsin. In this last medium the areas of the plates adjacent to the B. coli colonies were deeply colored. This diffusion was less marked with the other sample of dehydrated material and still less apparent in the standard Endo agar.

Staphylococcus aureus grew in small, regular, pink colonies on the dehydrated medium containing 0.1 part basic fuchsin and on the standard Endo agar. However, it was entirely inhibited on plates of the dehydrated medium which contained the higher percentage of fuchsin.

These same samples of dehydrated material also greatly inhibited the growth of the Shiga strain of B. dysenteriae. Plates inoculated with this culture developed such very fine colonies after an incubation period of eighteen hours that identification was difficult. If these plates were incubated for a longer period, they deepened so much in color that they were of little use in differentiation. However, plates of the routine medium and of the other sample of dehydrated medium which had been inoculated with the same amount of Shiga culture developed many small characteristic colonies in eighteen hours at 37° C.

The cultures of B. typhosus, B. paratyphosus A, B. paratyphosus B, and B. dysenteriae, except the Shiga strain, grew about equally well on all samples of the dehydrated medium and on the standard Endo agar. The only noticeable difference between the colonies on the various media was their color in reflected light. On the dehydrated medium all typhoid and dysentery colonies were slightly pink, while on the routine medium they were colorless. However, all these colonies were colorless to transmitted light regardless of the medium.

When mixtures of B. typhosus and B. coli (1:5, 1:10, 1:15, 1:20) and of B. dysenteriae Mt. Desert strain and B. coli (1:6, 1:12, 1:24, 1:30, 1:36) were plated on the two Endo media, the importance of the difference in the amount of diffusion of the dye was plainly shown. If the mixtures were highly diluted so that very few organisms were present in each loopful of suspension, B. typhosus and B. dysenteriæ could be isolated from all mixtures. With mixtures less highly diluted the color produced by the B. coli diffused throughout the entire plate of the dehydrated medium, and the typhoid and dysentery colonies were not apparent in the mixtures containing large numbers of B. coli. This was not true of the standard medium where the diffusion was less marked.

Organisms of the enteric-disease group were isolated from all of the thirty specimens of feces which were plated on both of the Endo agars. None of these specimens contained large numbers of B. coli, probably due to the fact that they had been kept in glycerin-salt solution. One positive fishing from each medium was tested by macroscopic agglutination. Variations in amount of agglutination occurred between the cultures grown on the different media, but these variations were not sufficiently marked to warrant any definite conclusion.

The dehydrated triple-sugar medium was first tested by comparing the reactions produced in eighteen hours by the growth of pure cultures in the dehydrated medium with the reaction produced by the same cultures in the standard medium. Table I gives the results of several such tests.

TABLE I

COMPARISON OF THE REACTIONS PRODUCED IN THE TWO TRIPLE-SUGAR MEDIA AFTER EIGHTEEN HOURS AT 37°

CULTURE	DEHYDRATED TRIPLE SUGAR	STANDARD TRIPLE SUGAR
Staphylococcus aureus	Acid slant	±
B. coli	+g	±g
B. typhosus Bender	±	+
B. paratyphosus A	±g	+g
B. paratyphosus B	±g	+g
B. dysenteriae Flexner	+	+
B. dysenteriae Mt. Desert	±	+
B. dysenteriae Shiga	± or acid along slant or line of inoculation in butt	+

+ = acid in butt only, no gas.
+g = acid and gas in butt.
± = acid in butt and slant, no gas.
±g = acid and gas in butt, acid slant.

All of the samples of the dehydrated medium gave the same results except the one containing phenol-red indicator. In this medium B. dysenteriæ Flexner produced an acid reaction in the slant as well as the butt, and B. paratyphosus B was the only culture to produce a characteristic triple-sugar reaction. The growth of all cultures on all samples of dehydrated triple sugar was poor. The reactions produced by freshly isolated cultures did not differ from those produced by the test cultures. A longer incubation period, from forty-two to sixty-six hours, increased the number of characteristic reactions of some of the cultures, but such an incubation period would be a great disadvantage even if the medium were entirely satisfactory otherwise.

The only differences in the compositions of the two triple-sugar media lay in the amount of beef extract and dextrose present. The standard medium contained 0.05 per cent dextrose and 0.3 per cent beef extract, while the dehydrated medium contained twice as much dextrose and no beef extract. By making small amounts of medium according to the formula used here, but varying the amounts of these two ingredients to correspond with the dehydrated medium, it was found that the test cultures produced many of the same reactions in a medium containing 0.1 per cent dextrose as in the dehy-

drated medium, and that the addition of beef extract had little effect except on the amount of growth produced. However, when 0.3 per cent beef extract was added to the dehydrated medium, not only was the growth improved, but many of the reactions produced by the test cultures became characteristic.

CONCLUSIONS

The dehydrated Endo-agar medium though less efficient for the isolation of organisms of the enteric-disease group than the standard Endo agar made according to the Robinson and Rettger modification, supports a good growth of all organisms except the Shiga strain of B. dysenteriae.

The dehydrated medium permits a larger amount of diffusion of the dye in the areas of the medium surrounding B. coli colonies than does the standard Endo agar. Of the various samples of the dehydrated medium the ones containing the larger amount of basic fuchsin show the more marked diffusion.

When positive specimens preserved in glycerin-salt solution are plated on the dehydrated medium, the amount of diffusion is not sufficient to obscure the typhoid or dysentery colonies.

The results of the comparison of the dehydrated Endo medium with the standard Endo medium indicate that the dehydrated medium while less efficient is nevertheless a usable medium for the isolation of organisms of the enteric-disease group with the exception of the Shiga strain of B. dysenteriae.

The dehydrated Krumwiede triple-sugar agar is not usable for the isolation and identification of organisms of this group. This medium appears to contain too high a percentage of dextrose and too little nutriment to produce a good growth and characteristic reactions when inoculated with organisms of the enteric-disease group.

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OXYTOCIC APPARATUS WITH TWELVE TUBES*

By E. P. Bugbee, M.D., and A. E. Simond, B.S., Detroit, Michigan

IN THE course of the work which led up to the separation of two active principles from the posterior lobe of the pituitary gland[1] several hundred oxytocic tests had to be made. An oxytocic apparatus on which two uterine muscles could be worked simultaneously, was not adequate to handle the great number of tests involved so another apparatus was arranged to work six muscles at a time. Even this apparatus was unable to handle the volume of tests and a new apparatus has been built which can work twelve uterine muscles simultaneously. It employs the same glass muscle tubes and air tubes as have been used for five years by Mr. L. W. Rowe of this laboratory. These in turn are a development from the earlier models described by Hamilton and Rowe in 1916.[2] The six tube and the twelve tube oxytocic apparatus were built for us by Eberbach and Son Company of Ann Arbor, Michigan. Both apparatus have been in use for several months and are very satisfactory. The twelve tube or "twin six" is shown in Fig. 1. An electrically heated water-bath maintains a temperature of 38° C. Partially submerged in the water stand twelve vertical muscle tubes in which the uterine muscles are suspended. Above the water-bath extends a bar which supports twelve upright rods. To the upright rods are clamped the writing levers which magnify the contractions of the muscles and record these contractions on the smoked paper of the kymograph.

The water-bath itself is a copper tank 37 inches long, 14 inches wide and 8 inches deep. In the front of the tank are two glass windows, 11½ inches long and 5¾ inches high. The back of the tank is lined with opal glass to act as a reflector as well as to improve the appearance. The tank is covered with insulating material to reduce loss of heat. The tank rests on a base plate of asbestos board supported by an angle iron frame and legs 4 inches long. Flush with the surface of the asbestos board are three electric heating units such as are employed in electric grills. The three heating units are alike and together carry 460 watts. The center unit carries current continuously but the two end units are intermittent in their action. They are controlled by Eberbach thermostatic regulators of the bimetallic type. In parallel with each thermostat there is a condenser to prevent arcing and an 8 C.P. 250 V. carbon lamp to act as a pilot light. The lamp is lighted when the current is not passing through the heating unit. The regulation is entirely satisfactory and the temperature of the water is maintained all day long with a fluctuation of only half a degree. The water tank holds fifty liters of water and the change in temperature of such a large volume of water takes place only very gradually.

*From the Medical Research Laboratory, Parke, Davis and Company, Detroit, Michigan. Received for publication, February 11, 1928.

The water tank has 13 holes or tubulatures in the bottom. These are of the proper size to accommodate No. 6 rubber stoppers. One hole carries the drain pipe for emptying the tank and the other twelve holes carry the drain tubes for emptying the vertical tubes in which the muscles are suspended. The muscle tubes are 7¼ inches long and 1¼ inches inside diameter, and their capacity is 150 c.c. A graduation is marked at the 100 c.c. level. In use they stand vertical and the upper end is open, Fig. 2. To the lower rounded end is attached a one-fourth inch glass tube which serves as a drain tube. Near the lower end is attached a side tube which serves as a filling tube.

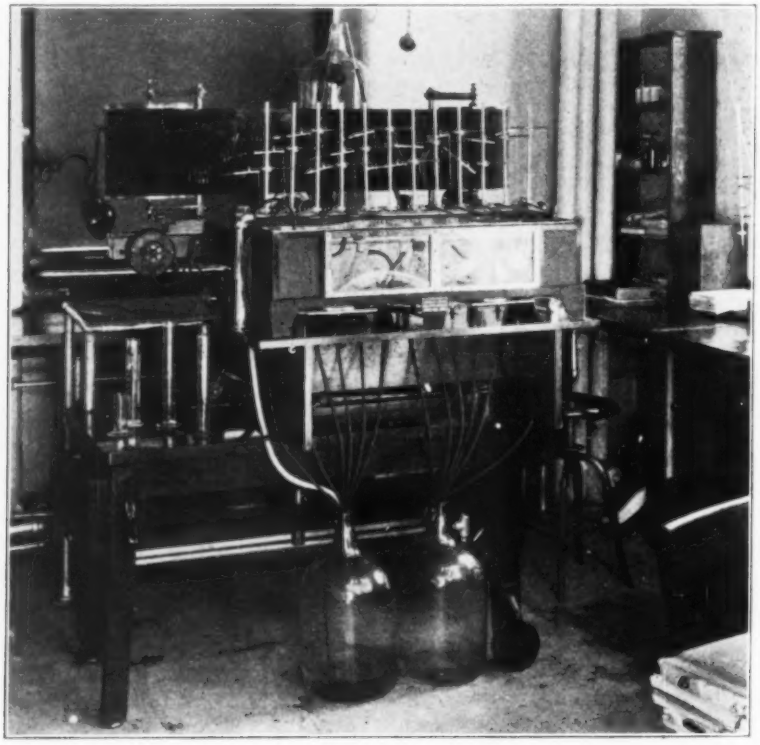

Fig. 1.—Twelve tube oxytocic apparatus making twelve records on kymograph.

The arrangement of the apparatus is quite similar to that described in detail by Dale and Laidlaw in 1912.[3] It differs only in some details from the apparatus described more recently by Pittinger and his associates,[4] Roth,[5] Hamilton and Rowe,[2] Eckler,[6] Burn and Dale,[7] Smith and McClosky,[8] Nelson,[9] and Swanson.[10]

The vertical muscle tube is filled to the 100 c.c. level with Locke's solution (Fig. 2). Air is bubbled through the solution constantly by means of a glass air tube which enters the muscle tube from above and extends downward so that the air aperture is nearly at the bottom of the Locke's solution. An L-shaped projection from the air tube furnishes a convenient anchor for the lower end of a strip of uterine muscle which is tied to it by a loop of

thread. The upper and movable end of the muscle is attached by a thread to the short arm of an Eberbach muscle lever. This lever multiplies the movement of the muscle about three times. It is counterweighted by attaching small weights to the longer lever arm.

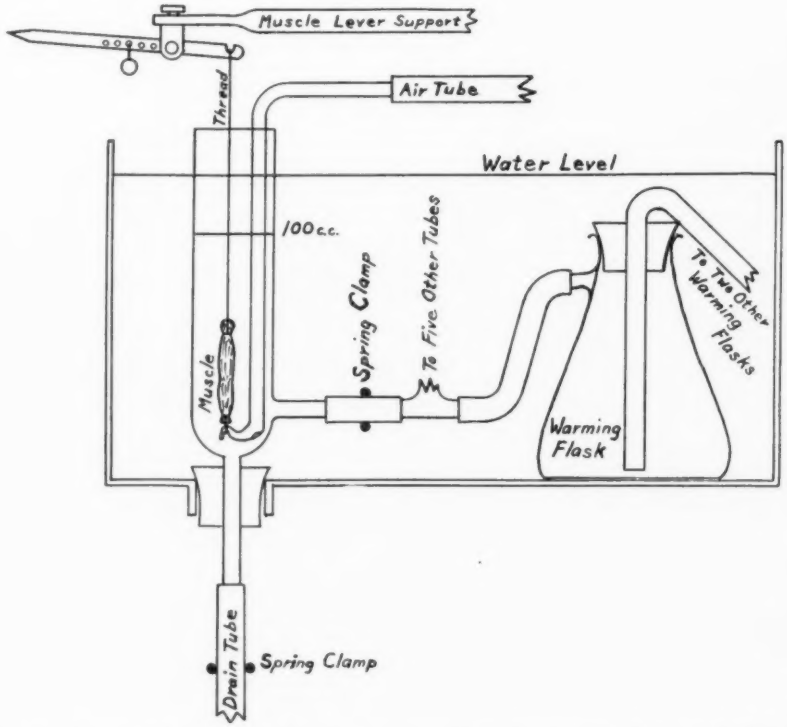

Fig. 2.—Muscle tube supported by rubber stopper in tubulature of water tank. Uterine muscle anchored to air tube and attached to muscle lever by a thread. Warming flask to supply Locke's solution at 38° C. for filling muscle tube.

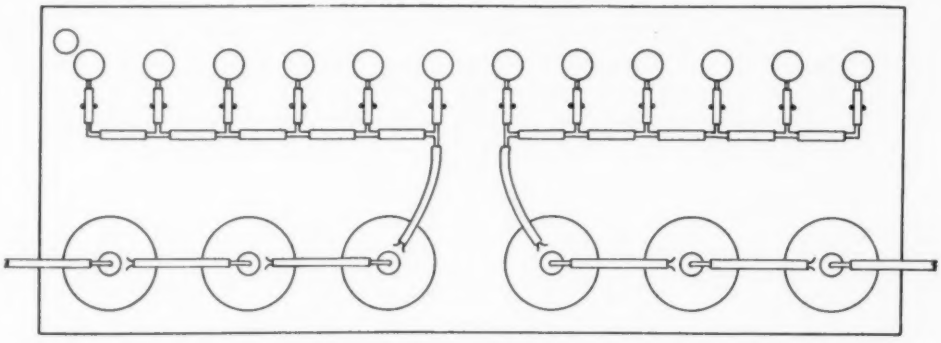

Fig. 3.—Diagram of 12 muscle tubes in two series, each of which is supplied with Locke's solution from 3 warming flasks.

The Locke's solution used in the muscle tube is preheated by running it through a series of three warming flasks immersed in the water-bath. The arrangement of the warming flasks and muscle tubes is shown in Figs. 2 and

3. The muscle tubes are emptied by operating the spring clamp or pinchcock on the drain tube and can be refilled with fresh Locke's solution by operating the clamp on the filling tube. This latter procedure requires immersion of the hand in the water-bath which serves the useful purpose of agitating the water to keep it of uniform temperature.

The Locke's solution is supplied from an elevated 20 liter bottle. The air for oxygenating the Locke's solution in the muscle tubes is obtained from the low pressure compressed air line. A header on the air line is equipped with twelve needle valves so that the flow of air through each muscle tube can be regulated independently of the others. The air is bubbled through wash bottles containing a 2 per cent solution of sodium bicarbonate as recommended by Smith and McClosky.[8]

Our thanks are extended to Mr. L. T. Clark, Junior Director of the Medical Research Laboratories, for suggesting the need of a multiple test apparatus and for his interest and help in the design and subsequent use.

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A RAPID QUANTITATIVE METHOD FOR THE DETERMINATION OF ACETONE AND DIACETIC ACID IN URINE*

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THE method described in this paper for the quantitative determination of urinary acetone and diacetic acid was designed for use in laboratories where a rapid and standardized procedure is necessary. It is at present in use in this laboratory for the analysis of samples which give doubtful or positive results by the qualitative test recently described in this Journal.[1]

Like the qualitative test, this method is based upon the reaction which takes place between acetone and salicylic aldehyde in alkaline solution. The product which is formed has an intensity of color proportional to the concentration of acetone in the solution. The method is essentially the same as that described by Behre and Benedict[2] for the determination of all the acetone bodies in both blood and urine. The procedure, however, has been simplified as much as possible for the present purpose.

A distillation is required, as no satisfactory method has been found for carrying out the reaction in the urine directly. For the sake of accuracy the volumetric measurements and the distillation should be performed with care, but no elaborate technic is involved. The entire determination can be completed in ten or twelve minutes.

The relative amounts of the reagents used are slightly different from those called for in the original method. A definite amount of urine (10 c.c., unless this amount is not available) is distilled to an equal volume. Duplicate determinations may be made on the distillate. Diacetic acid is converted to acetone during the distillation, and determined as such, with the preformed acetone, in the distillate.

A set of permanent color standards has been devised, in order to obviate the necessity of keeping standard acetone solutions on hand. These standards involve the use of a side-to-side, test tube comparison, and for this it is of great importance that the tubes which are used have a diameter accurately measured to correspond with the diameter of the standard tubes. Such a reading is necessarily inaccurate compared with a reading made in a colorimeter, but if carefully standardized tubes are used an accuracy is obtained which is quite sufficient for clinical work. Comparison in a colorimeter is impractical when the permanent standards are used on account of the rapid increase of color which takes place in the final process, as the solution cools. The side-to-side comparison is made before any cooling has taken place.

*From The Biochemical Laboratory of the Union Central Life Insurance Company, Cincinnati.

Received for publication, March 5, 1928.

The present paper does not include a description of the determination of β-hydroxybutyric acid, but the oxidation of this acid may be carried out as described in the earlier paper[2] and the resulting acetone determined by the present method.

THE METHOD

1. *Apparatus.*—

Water-cooled condenser (250 mm. from shoulder to shoulder, straight tube).

Distilling flask (125 or 200 c.c. capacity).

Glass delivery tube (see Fig. 1) to fit with cork stopper onto end of condenser, and drawn out at the other end to reach into receiving tube. Curved glass "adapters" may be used for this purpose.

Receiving tube, graduated to 10 c.c., and of a size to allow the delivery tube to reach to the bottom.

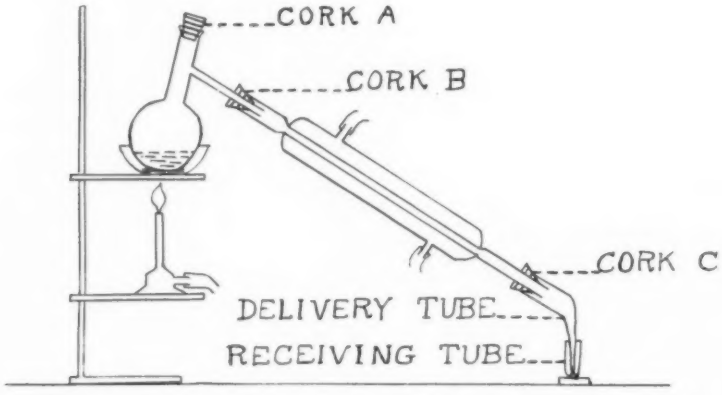

Fig. 1.—Distillation apparatus for the quantitative determination of acetone and diacetic acid.

Tubes for the determination, of exactly the same diameter as that of the standard tubes. (The standardized tubes used for the sulphosalicylic acid albumin determination, obtained from the Fales Chemical Company, are good for this purpose.)

Sand-bath and Water-bath.

Set of standards. Directions for making these are given below.

2. *Reagents.*—

Sulphuric acid, concentrated acid added to an equal volume of distilled water.

Sodium hydroxide, 32 per cent solution.

Salicylic aldehyde, from Eimer and Amend, labelled "Acid Salicylous, Synthetic (Salicylic Aldehyde)." *It is important to use the same make of salicyl aldehyde that was used in making the standards,* as different makes vary greatly in the intensity of color which they give in this reaction. This reagent should be kept in a dark-colored, glass-stoppered bottle. It may be added from a dark-colored dropping bottle.

3. *Distillation for the Determination of Acetone and Diacetic Acid.*—The apparatus for distillation is first made ready, the water connection turned on and the glass tip of the delivery tube on the end of the condenser introduced into the receiving tube which contains a few drops of distilled water, so that the tip of the delivery tube dips below the surface of a minimum amount of water (see Fig. 1).

Ten c.c. of the urine to be tested are measured with a pipette into the distilling flask, and about ten drops of the sulphuric acid and 25 to 30 c.c. of distilled water are added. The flask is *tightly* stoppered with a good cork stopper (cork A, Fig. 1). The stopper and the neck of the flask should be thoroughly dry, and the stopper should fit perfectly, so that none of the volatile acetone can escape.

The flask is then connected with the water-cooled condenser by means of a cork stopper fitted onto the arm of the flask (Fig. 1, Cork B). This connection should also be very tight. It is very important that all of the corks are dry and tightly fitting. Several corks of the right sizes (A and B) may be kept in reserve, with holes bored in B, so that dry ones may always be used. Rubber connections must never be used in places where the acetone may come in contact with them, as rubber reacts to give undue color in the determination.

The sand-bath is arranged under the flask, the burner lighted under it and distillation carried on. The boiling should not be so violent that the urine goes over without distillation, but a good flame should be used to prevent sucking back. If this should occur the delivery tube at the end of the condenser may be loosened a little to let in air at this point.

When the volume of the distillate has almost reached the 10 c.c. mark the delivery tube is disconnected from the condenser and blown out into the receiving tube. It may also be washed down with a few drops of water and drained into the receiving tube. The volume is made to just 10 c.c. The burner is turned off *after* the delivery tube has been disconnected. The receiving tube is then inverted several times to mix thoroughly.

If 10 c.c. of urine are not available for the distillation, a smaller volume may be used and distilled to an equal volume (marked on the receiving tube). If as little as 5 c.c. are used, and distilled to 5 c.c., the recovery of acetone in the distillate is almost, but not quite, complete.

4. *Determination of Acetone in the Distillate.*—Two c.c. of the distillate are transferred to one of the regular acetone tubes with a 2 c.c. pipette, 2 c.c. of 32 per cent sodium hydroxide are added from a burette, and 2 drops of salicylic aldehyde (Emer and Amend) from a dropping bottle. Not more than 2 drops are to be added; if more are dropped in by mistake this portion of the distillate should be discarded and 2 c.c. more taken. The tube is shaken from side to side and put into a boiling water-bath for three minutes. Shortly after it has been put in, it should be shaken again from side to side so that all of the salicylic aldehyde is dissolved and the contents of the tube well mixed. The tube may be left in the bath longer than three minutes, but as soon as it

is removed it must be compared with the standards. The hot water on the outside of the tube is wiped off and the comparison made *immediately*. The solution darkens rapidly when allowed to cool, and the reading will be too high if made even two minutes after the tube has been taken from the bath. If there is an unavoidable delay in making the reading, the tube may be returned to the water-bath and left for several minutes, after which the increase in color has disappeared, and the reading may again be made as soon as the tube is removed from the bath. The tube should not be left in the bath for more than ten minutes at most, however.

The reading consists of a side-to-side comparison with the standard tubes, by transmitted light. The percentage of acetone, from diacetic acid and acetone, in the original urine, is read directly from the label on the standard tube which most nearly matches the color given by the distillate.

If the acetone content is greater than that represented by the highest standard (0.01 per cent), the distillate may be diluted and the colorimetric determination repeated.

The presence of formaldehyde in the urine interferes to a certain extent with the full development of color in this reaction. If a preservative has been used, such as urotropin, which develops formaldehyde, the reaction of the urine should be made almost neutral, or very slightly acid, before distillation, the addition of sulphuric acid being omitted.

5. *Standards for the Color Comparison.*—These standards were made to match the color produced in acetone solutions treated by the colorimetric method described above, using Eimer and Amend's salicylic aldehyde. The acetone content of the solutions used for comparison was determined by iodometric titration.

We have kept these standards for almost a year in this laboratory, at the present time, without finding any change in their color. It is best to keep them in sealed tubes. Tubes of the standard size may be drawn out at the end, the standard solution introduced and the tubes sealed off.

The formulas for the standards to match various percentages of acetone follow. Only c.p. chemicals (crystals) should be used.

Blank (color given by the reagents without acetone).—Four hundredths gm. potassium bichromate + 0.06 gm. potassium chromate, made to 100 c.c. with distilled water.

0.001 per cent (0.001 gm. acetone per 100 c.c. urine).—One-tenth gm. potassium bichromate + 0.6 gm. cobalt chloride, to 100 c.c. with distilled water.

0.002 per cent (0.002 gm. acetone per 100 c.c. urine).—Thirteen hundredths gm. potassium bichromate + 1.32 gm. cobalt chloride, to 100 c.c. with distilled water.

0.003 per cent (0.003 gm. acetone per 100 c.c. urine).—Two-tenths gm. potassium bichromate + 2.0 gm. cobalt chloride, to 100 c.c. with distilled water.

0.004 per cent (0.004 gm. acetone per 100 c.c. urine).—One and three-tenths gm. potassium bichromate + 1.3 gm. cobalt chloride, to 100 c.c. with distilled water.

0.005 per cent (0.005 gm. acetone per 100 c.c. urine).—One and seven-tenths gm. potassium bichromate + 1.7 gm. cobalt chloride, to 100 c.c. with distilled water.

0.0075 per cent (0.0075 gm. acetone per 100 c.c. urine).—Three gm. potassium bichromate + 3.0 gm. cobalt chloride, to 100 c.c. with distilled water.

0.01 per cent (0.01 gm. acetone per 100 c.c. urine).—Three and eight-tenths gm. potassium bichromate + 3.8 gm. cobalt chloride, to 100 c.c. with distilled water.

SUMMARY

A method is described for the quantitative determination of urinary acetone and diacetic acid, suitable for use in clinical laboratories.

REFERENCES

[1]Behre, J. A.: Jour. Lab. and Clin. Med., May, 1928, xiii, 770.
[2]Behre, J. A., and Benedict, S. R.: Jour. Biol. Chem., 1926, lxx, 487.

A COLORIMETRIC METHOD FOR THE DETERMINATION OF BILE ACIDS IN URINE*

By Ichiro Katayama, M.D., New York City

THE lack of a satisfactory method for bile acids in urine has been an impediment to a comprehensive study of bile acid metabolism in pathologic states in man. The elaborate technic utilized by Schmidt and his coworkers for the bile acids of bile and urine of experimental animals are not readily applicable in clinical laboratories. The principles of the method proposed by Szilárd[1] for the estimation of bile acids in blood have been adapted to analysis of urine. In order to separate the bile acids from interfering substances, advantage has been taken of the observation made by Tengström,[2] Schmidt and Merrill[3] that bile acids are precipitated from solution by saturating the solution with magnesium sulphate.

METHOD

An alcoholic solution of the bile acids of the urine, prepared by adding 50 c.c. of absolute alcohol to 10 c.c. of urine, is heated in a boiling water-bath to effect solution of the bile acids and to coagulate the proteins. This is filtered and the filtrate is evaporated at about 70° C. to dryness in vacuo. The residue is dissolved in about 15 c.c. distilled water and the bile acids are precipitated from their aqueous solution by the addition of solid magnesium sulphate to saturation. After standing in the ice box overnight, the residue is filtered on the best quality filter paper, and then washed with small quantities of a cold saturated solution of magnesium sulphate. After the filter paper has been dried completely, the bile acids are extracted overnight with 80 c.c. of absolute alcohol. This filtrate is evaporated in vacuo to 10 c.c. and 200 c.c. of absolute ether are added to the residue. This mixture is allowed to stand overnight. The bile acids are precipitated by this alcohol ether mixture. The supernatant fluid is removed by siphon without disturbing the precipitate. When entirely dry, the precipitate is dissolved in 5 c.c. of water, transferred to a 15 c.c. centrifuge tube, and 0.3 c.c. of 1 per cent ferric chloride solution is added and the solution thoroughly mixed. On standing in the

*From the Department of the Laboratories, New York Post-Graduate Medical School and Hospital.

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incubator at 38° C. overnight the bile acids combine with the ferric chloride and are precipitated as ferric salts. The mixture is centrifuged and the supernatant fluid removed by siphonage. After the precipitate has been washed twice with water, it is dissolved in 5 c.c. of absolute alcohol and quantitatively transferred to a test tube, using 3 c.c. absolute alcohol. To this test tube 5 c.c. of chloroform and 5 c.c. of sulphosalicylic acid reagent are added. After thoroughly mixing, the stoppered tube is permitted to stand in a bath at 40° to 50° C. with occasional shaking for two to three hours, i.e., until the purple color in the upper layer has reached its maximum intensity.

A stock solution of 0.1 per cent glycocholic acid may be kept for about one week. From this stock solution a series of four standards for comparison are prepared by pipetting into 15 c.c. conical centrifuge tubes 2.0 c.c., 1.5 c.c., 1.0 c.c., and 0.5 c.c., respectively, of the stock solution. The contents of each tube is diluted to 5 c.c. and to this solution 0.3 c.c. of the ferric chloride reagent are added. The stoppered tubes containing the standard solutions are incubated at about 40° C. overnight. From this point the procedure for the development of the color in the standards is identical with that for the unknown. There are available four standards for comparison in the colorimeter, containing 2.0 mg., 1.5 mg., 1.0 mg., and 0.5 mg. of glycocholic acid.

To obtain the most satisfactory results it has been found advisable to keep the colorimetric reading within a narrow range, about 15 mm. This is accomplished by a comparison of the unknown with the standard most closely approximating it in color intensity. A sample from the upper colored layer of both standard and unknown is pipetted into the colorimeter cups for comparison.

Calculation: S = mg. of bile acid in the standard used for comparison.

$$\frac{15}{R} \times S \times \frac{\text{vol. of urine}}{\text{used urine vol.}} = \text{mg. of bile acids as glycocholic acid per volume of urine.}$$

Reagents.—Ferric chloride: 1 per cent of ferric chloride containing 0.05 per cent of concentrated hydrochloric acid.

Sulphosalicylic acid: 8 grams of sulphosalicylic acid in 1,000 c.c. of 0.01 N hydrochloric acid.

A satisfactory recovery of the bile acids in aqueous solution and of bile acids added to urine is indicated by the data in Tables I and II.

TABLE I

RECOVERY OF TAUROCHOLIC AND GLYCOCHOLIC ACID IN AQUEOUS SOLUTION

BILE ACID	THEORETIC MG. PER 100 C.C.	FOUND	RECOVERY PER CENT
Glycocholic	15.0	14.3	95
Glycocholic	10.0	9.5	95
Taurocholic	15.0	15.1	100
Taurocholic	10.0	9.9	99
		Average recovery	97.5

TABLE II

RECOVERY OF TAUROCHOLIC AND GLYCOCHOLIC ACIDS FROM URINE

PRESENT IN URINE	BILE ACID ADDED MG. PER 100 C.C.	FOUND	RECOVERY PER CENT
5.0	15 glycocholic	19.7	98
0.0	10 glycocholic	9.3	93
5.0	15 taurocholic	20.1	100
0.0	10 taurocholic	9.8	98
		Average recovery	97.5

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[1]Szilárd, P.: Biochem. Ztschr., 1926, clxxiii, 440.
[2]Tengström, S.: Ztschr. f. physiol. Chem., 1904, xli, 210.
[3]Schmidt, C. L. A., and Merrill, J. A.: Jour. Biol. Chem., 1923-24, lviii, 601.

A NOTE ON THE TECHNIC FOR STAINING THE NUCLEAR MATERIAL IN BLASTOMYCES*

BY EVERETT S. SANDERSON, PH.D., CHARLOTTESVILLE, VIRGINIA

WHILE engaged last year in the making and assembling of photographs from a case of systemic blastomycosis admitted to the University of

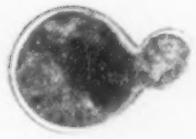

Fig. 1.

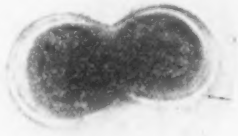

Fig. 2.

Fig. 3.

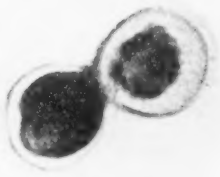

Fig. 4.

Blastomyces in the yeast stage stained with hematoxylin and showing the arrangement of nuclear material. From slant agar cultures three to five days old. Oil immersion. x 1500. Figs. 1, 2, 3, and 4.—Various stages in the budding process. Fig. 5.—An unstained organism for comparison. Figs. 6 and 7.—Transformation from yeast stage to mycelian form; unstained.

*From the Department of Pathology and Bacteriology, University of Virginia.
Received for publication, February 5, 1928.

Virginia Hospital, which were used as a part of the exhibit at the annual meeting of the American Medical Association,* it was felt that photographs of hematoxylin-stained organisms, to bring out the nuclear material during the process of budding, would be of added interest. It was found, however, that when smears of the blastomycees were dried on slides and stained in the usual way the thick outer capsule caused shrinking and distortion to such a degree that they were entirely unsatisfactory for photographic purposes. Several

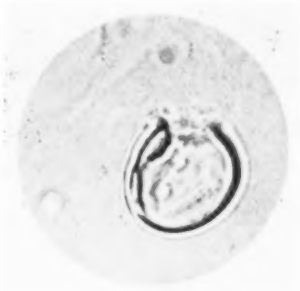

Fig. 5.

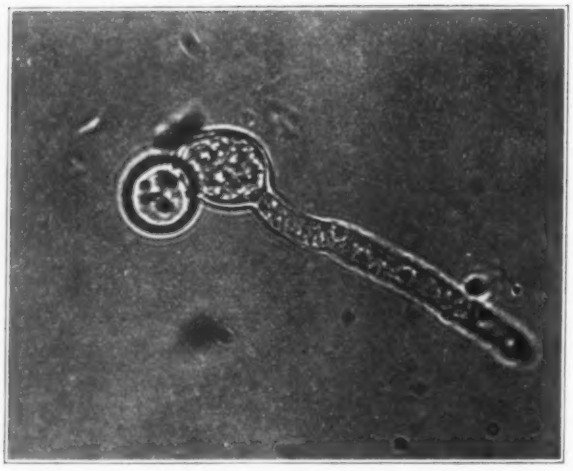

Fig. 6.

methods were tried to alleviate this difficulty, and the one here described gave very satisfactory results.

The organisms in the yeast, or budding stage, were washed off agar slants with water and then fixed by boiling in a small centrifuge tube for from thirty to fifty seconds. The emulsion was centrifugated, the supernatant fluid discarded, and the organisms resuspended in from one to two cubic centimeters of hematoxylin and stained for from two hours to overnight, depending upon the strength of the stain. At the end of this period the excess dye was removed by alternate centrifugation and washing in tap water, and

*In collaboration with Dr. D. C. Smith of the Department of Dermatology and Syphilology, University of Virginia.

to the last washing a trace of ammonia added to bring out the desired color. If permanent slides are desired, the organisms can be mounted in a glycerin jelly. Figs. 1 to 5 show the results to be had by this method.

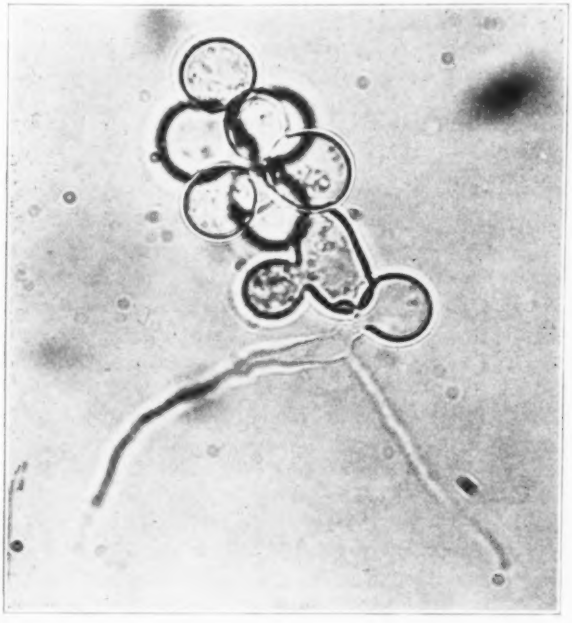

Fig. 7.

Two other photographs of unstained organisms are included to show the transformation from yeast type to mycelian form; the former being the one usually observed in tissues, while the latter is responsible for the "cottony" growth seen in cultures.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

LABORATORY TECHNIC

IMMUNITY: Experimental Immunization With Bacteria Detoxicated by Gold Chloride, Osman, M. B. Jour. Immunol., 1927, xiii, 3, 243.

Detoxication was carried out as described by Sanfilippo, by suspending growths of the various organisms in a 1 per cent solution of gold chloride. Organisms such as B. typhosus, grown on solid media, were emulsified in a known quantity of sterile distilled water to which an equal volume of 2 per cent gold chloride was added. In the case of streptococci and pneumococci which were grown in a veal extract phosphate bouillon, the organisms were deposited by centrifuging; the supernatant bouillon was completely removed (to avoid any formation of a precipitate on the addition of the gold solution) and the growth was then emulsified in distilled water to which 2 per cent gold chloride was added, as before. Immediately after the addition of the chemical, the organisms tended to agglutinate but the clumps could be broken up by shaking, thus allowing the gold salt to act uniformly. The treatment was allowed to proceed for one hour. The emulsions were then centrifuged and the deposit was washed with sterile distilled water. Clumps were again broken up to ensure thorough washing, which was repeated three times. After the third washing, the organisms were suspended in the minimum amount of distilled water.

For purposes of dosage in immunizing animals, the number of organisms per cubic centimeter of the suspensions was estimated by means of Brown's opacity standards. When large doses were to be injected, dense emulsions were made up and their concentration was computed by preparing a 1:10 dilution whose opacity fell within the range of Brown's standards. The number of organisms in the original suspension could then be estimated and the required dose given in small bulk.

In immunizing animals, all injections were given intravenously.

Gold chloride exerts a marked detoxicating effect on various bacteria.

The power of such detoxicated bacteria to stimulate agglutinin production is slight.

Animals immunized with such detoxicated organisms tolerate large doses of live organisms.

By immunizing with a large dose of detoxicated organisms, followed after a week by a large dose of live organisms, an agglutinating antiserum of high titer can be readily obtained.

FECES: A Rapid Method for the Detection of Ova and Cysts of Intestinal Parasites, Rivas, D. Jour. Trop. Med., 1928, viii, 1, 63.

1. Place 1 to 2 gm. feces in a medium or large sized test tube and add 5 c.c. of 5 per cent acetic acid for each 5 gm. of feces.

2. Stopper tube and shake until suspension is homogeneous.

3. Allow to stand one minute for coarse particles to settle.

4. Remove supernatant fluid with a pipette or, if necessary, filter through double layer of cheesecloth.

5. Place 5 c.c. of filtrate in centrifuge tube, add an equal volume of ether; stopper and shake horizontally until homogeneous (a few seconds).

6. Centrifuge for one to two minutes.

7. Pour off all but the sediment (which may be very scanty).

8. Mount and examine the sediment.

FECES EXAMINATION: Technic of Examination of Feces for Amebas and Protozoa. Albert, H. Arch. Path. and Lab. Med., 1927, iii, 227.

1. Iodine-Eosin stain of fresh material. A drop of physiologic sodium chloride solution and one of iodine-eosin stain are placed near together on a slide, but not touching. A round applicator stick or a toothpick is smeared with the feces, and rolled in the drop of physiologic sodium chloride solution and then in the drop of iodine-eosin. A single cover-slip is placed on both drops, half the material under it being stained and the other half unstained. The unstained portion should be examined first for living flagellates and active amebas. In the stained portion the protozoan cysts stand out as bright spherules against the pink background and soon become tinged with the iodine to varying tones of yellow, with the nuclei becoming clearly defined as the iodine penetrates. If glycogen is present in the cysts, it becomes light or dark brown.

Iodine-eosin stain consists of: saturated aqueous solution of eosin in physiologic sodium chloride solution, 2 parts; 5 per cent solution of potassium iodide in physiologic sodium chloride solution saturated with iodine, 1 part; physiologic sodium chloride solution, 2 parts.

The proportion of iodine solution used may be modified to advantage by adding a slight excess of that given in the formula, if the nuclei do not appear after a few moments' application of the stain. The stain should be made up each day from the stock ingredients.

If no bright spherules stand out against the pink background or no other evidence of protozoa is found in two preparations examined, the specimen is reported as negative. If bright spherules (cysts) or living protozoa in the vegetative stage are found, several smears are made and stained with hematoxylin, using the following method.

2. Hematoxylin stain of fixed material. A smear is made on a slide which has previously been thoroughly cleaned in alcohol-ether and flamed. If the fecal material is too dry, it should be moistened slightly with physiologic sodium chloride solution and a thin smear made with the applicator stick or the flat side of a toothpick or by using the edge of another slide or a cover-slip. It should then be immersed directly in Schaudinn's fixing fluid without allowing the slide to become dry. The following steps are used in fixing and staining the preparation:

Schaudinn's fluid (2 parts saturated aqueous mercuric chloride in physiologic sodium chloride solution, 1 part absolute or 95 per cent alcohol; 4 c.c. glacial acetic acid is added to 90 c.c. of the mixture on using) (freshly prepared each time) is heated to from 56 to 60° C. for 10 minutes.

70 per cent alcohol tinged with Gram's iodine	5 minutes
70 per cent alcohol	5 minutes
50 per cent alcohol	5 minutes
Tap water	2 minutes
2 per cent iron alum* aqueous solution	5 to 12 hours
or heated to 30° C.	10 minutes to 1 hour
Tap water—rinse	1 minute
5 per cent hematoxylin† aqueous solution	12 to 18 hours
or heated to 30° C.	10 minutes to 1 hour
Tap water	1 minute

Differentiate in 1 or 2 per cent iron alum with careful watching under microscope. (Do not allow to dry!) (After placing in alum solution about a minute, wash in water, then make trial examination.)

Wash in running water	10 minutes
50 per cent alcohol	10 minutes
70 per cent alcohol	5 minutes

*Use only violet crystals of iron alum. Reject yellowish powder.
†Use American hematoxylin, standardized, white crystals only.

90 per cent alcohol	5 minutes
100 per cent alcohol	5 minutes
Xylol	5 minutes

The preparation is then mounted in balsam and covered. Examination is made preferably with a binocular microscope using the oil immersion lens.

SPIROCHETA PALLIDA: Method for Demonstration in Single Sections, Dieterle, R. R. Arch. Neurol. and Psychiat., 1927, xviii, 73.

Reagents:

Uranium nitrate	5 gm.
70 per cent alcohol	500 c.c.
Gum mastic solution:	
Gum mastic	10 gm.
Absolute alcohol	100 c.c.

Allow to stand with frequent shaking for three days or until dissolved. Filter through three-fold filter.

Silver nitrate solution:
1 per cent aqueous (protect from light)

Developing solution:

Hydroquinone	1.5 gm.
Sodium sulphite	0.25 gm.
Merck's Blue Label Formaldehyde (neutral)	10 c.c.
Acetone	10 c.c.
Pyridine	10 c.c.
Water to make	90 c.c.

Mix, dissolve, and add 10 c.c. of 10 per cent alcoholic gum mastic solution to make the mixture "milky."

Method: (Applicable to frozen, paraffin, or celloidin sections after formalin fixation).

1. Place sections in the alcoholic uranium solution at 55° C. for one hour.

2. Wash for a moment in distilled water.

3. Pass through 96 per cent alcohol.

4. Handling the sections individually, place them in the gum-mastic solution for about thirty seconds. Then immerse for an instant in 96 per cent alcohol.

5. Transfer to distilled water.

6. Silver nitrate solution one to six hours at 55° C. protecting from light (overnight at 37° C.).

7. Wash for a moment in distilled water.

8. Place in developing solution five to fifteen minutes.

9. Rinse in distilled water.

10. Pass through 96 per cent alcohol and acetone, several changes.

11. Xylene and mount in balsam.

Frozen sections should be made after twelve hours' fixation in 10 per cent formaldehyde. After passing celloidin sections through acetone, and paraffin sections through Xylene and alcohol, proceed with Step 1.

Smears from a primary lesion or tissue emulsion are placed in 10 per cent formalin in 95 per cent alcohol (10:90) for one hour, rinsed in water and then started with Step 1.

The spirochetes are golden-yellow to golden-brown and are best photographed with a red filter, using a light blue for histologic detail.

PARESIS: Types of Therapeutic Response Observed in the Malaria Treatment of General Paralysis, Kirby, G. H. and Bunker, H. A. Am. Jour. Psych., 1926, vi, 2, 205.

It is possible to divide into three major groups 93 cases of general paralysis who were given the malaria treatment between June 15, 1923, and December 15, 1925, and who survived the period of treatment by more than two months: (1) Those in whom the treatment has apparently been without any effect whatever, 13 in number; (2) those in whom the results attributable to treatment have been of a more or less temporary character, 15 in number; and (3) those in whom the therapeutic influence, whatever its degree, has persisted essentially unmodified up to the present time, 65 in number. The last two groups are, of course, further divisible according to the degree of temporary or "permanent" improvement. Provisionally included among the 65 "permanent" results are 18 patients who, though they have exhibited no improvement, have manifested so far, after various periods extending up to three years, no evidence of retrogression.

The better the therapeutic result obtained, the more likely it is to be of a more or less enduring character; for the ratio of those who evidenced a subsequent decline to those who have so far preserved the status quo is as 8:2 among the only slightly improved cases, as 6:9 among the considerably improved, and as 1:36 among the full remissions.

Of 41 patients followed for more than a year, who received no further antisyphilitic treatment during that period, a well marked modification in the strength of the spinal fluid Wassermann took place in 13, or in 32 per cent (in 8 of whom the reaction became completely negative); in 11, or 26 per cent, the Wassermann reaction was definitely modified; in 17, or in 42 per cent, it remained unchanged. No very striking correlation between clinical status and spinal fluid findings was present, save that six of the eight cases to become Wassermann-negative had attained full remissions. In the eleven other full remissions in this group the Wassermann reaction in the spinal fluid was unchanged or only slightly modified. The Wassermann reaction in the blood remained essentially unchanged in 28 of the 36 patients who were positive prior to treatment (or in 78 per cent); in only six cases did the blood become negative with an alcoholic antigen and in only two with a cholesterinized antigen as well.

Patients of the "manic" type of general paralysis exhibited by far the greatest tendency to a favorable response to treatment. Of 16 "manic" cases in this series, 13 achieved a "permanent" result and 12 of these a full remission. Of 48 patients of the simple dementing type, 30 achieved a permanent result, but 13 of these belong in the stationary and unimproved group, and only seven attained a full remission.

The total duration of the various physical and mental symptoms referable to general paralysis averaged, at one extreme, twenty-seven months in the cases upon whom treatment was without influence, and at the other, sixteen months in the patients who achieved full remissions. There was little difference in this respect between the "temporary" group and the "permanent" group, as a whole.

In the 7 out of 12 patients who did not respond to treatment a more or less progressive loss of weight took place subsequent to treatment; 80 per cent of the "temporary" cases eventually lost much of the weight which they had previously gained; 48 per cent of the "permanent" cases registered a more or less progressive gain in weight without subsequent loss. In the case of temporary gain in weight, all three groups of patients made a gain of equal magnitude, amounting to about 16 per cent of the pretreatment weight, on the average; but the cases uninfluenced by treatment and the "temporary" cases subsequently lost about 85 per cent of this gain whereas the "permanent" cases lost only 48 per cent. When this posttreatment gain in weight was sustained without subsequent loss, the amount of this gain was likewise about 17 per cent of the pretreatment weight, on the average.

Among the clinical factors which seem to play a part in the results obtained by the malaria treatment, the so-called clinical type of general paralysis appears to rank first in importance, the behavior of the body weight subsequent to treatment second, and the duration of the symptoms prior to treatment third.

ACIDOSIS: A Reliable Clinical Method for Estimating Acidosis, Breed, L. M. Jour. Am. Med. Assn., October 30, 1926, lxxxvii, 1478.

Technic: One cubic centimeter of freshly drawn whole blood is added to 15 c.c. of acetone free methyl alcohol in a 25 c.c. volumetric flask. The mixture is shaken thoroughly and filtered into a beaker of 100 c.c. capacity. A few drops of phenolphthalein is added as an indicator to the filtrate which is then evaporated, just to dryness, over a water bath. The residue is redissolved in 10 c.c. of distilled water, and compared with 10 c.c. of phenolphthalein solution in the same sized beaker. If the color matches, one may know instantly that acidosis is not present, and likewise, if no color is evident, that acidosis is present. For exact figures, the estimation is as follows:

If the color matches the standard and remains for six hours it is marked: x.x.x.
If it remains for five hours it is marked: x.x.·.
If it remains for four hours it is marked: x.x.–.
If it remains for three hours it is marked: x.·.–.
If it remains for two hours it is marked: x.–.–.
If it remains for one hour it is marked: ·.–.–.
If it has no color at all it is marked: –.–.–.

VALUES FOR COMPARING CARBONATE CONTENT WITH PERCENTAGE BY VOLUME OF CARBON DIOXIDE

CARBONATE CONTENT	CARBON DIOXIDE PERCENTAGE BY VOLUME
Color for six hours, x.x.x.	over 72
Color for five hours, x.x.·.	67 — 71
Color for four hours, x.x.–.	63 — 67
Color for three hours, x.·.–.	61 — 63
Color for two hours, x. .–.	55 — 60
Color for one hour, ·.–.–.	44 — 48
No color, –.–.–.	less than 37

The distilled water must have been titrated with phenolphthalein and kept in a room free from acid fumes, and the redissolved filtrate must be placed in the same room and compared with the standard every hour for completing the estimation by comparing with the table of values.

The phenolphthalein standard solution is made by dissolving 1 c.c. of Hynson and Westcott phenolsulphonephthalein in 1,000 c.c. of distilled water. This will keep indefinitely.

BLOOD GROUPING: A Simple Method of Testing for Blood Compatibility, Felsen, J. Arch. Path. and Lab. Med., 1927, iv, 4, 552.

Blood is collected in two capillary tubes, one of which contains a few grains of powdered sodium citrate, from the recipient (R) and the prospective donors (D 1-2-3, etc.).

The citrated capillary tube is rotated and inverted 20 times to insure thorough mixing and, after the blood is allowed to gravitate to one end, this end is plugged with soft wax or paraffin. Both the plain and citrated tubes are inserted in a cork bored ⅔ its length and the cells and plasma and clot and serum separated by centrifuging or allowing to stand twenty minutes. Scratch marks are made with a file just above the wax plug and at the juncture of cells and plasma and the tube broken the tip containing the wax plug being discarded. There are now four tubes for each person: (1) citrated cells; (2) plasma; (3) clot; (4) serum.

Using a fine capillary rod (a separate one for each person), place a small drop of recipient's cells and donor's serum on a clean glass slide, and a similar drop of donor's cells and recipient's serum, and mix. Read for agglutination or hemolysis with the naked eye (fifteen minute time limit).

Time is saved if only those donors theoretically compatible, are tested. Grouping is done with the citrated cells and known group sera.

The serum of compatible donors (from the uncitrated capillary tube) is used for a Kahn test and one drop used for an intracutaneous test on the recipient.

REVIEWS

Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.

*The Erythrocyte and the Action of Simple Hemolysins**

THE increasing specialization in all fields of medicine, clinical or experimental, and the rapidity with which developments occur make it practically impossible for any one author to deal adequately with current advances in knowledge. To meet this situation the textbook is supplemented by the monograph.

Especially is this true of those branches or divisions of the medical art which are the subject of experimental investigation.

This volume is a monographic contribution in a special field concerned with the action of simple hemolysins, presenting not only the researches of the author but also a comprehensive survey of all the related contributions which have been made upon the subject.

The book is divided into two sections. The first is devoted to the morphology, chemistry, and structure of erythrocytes in general and comprises, in 85 pages, a very complete survey of the literature on the question and the arguments for and against the various theories advanced concerning particularly the morphology and structure of erythrocytes.

In the second part of the book (95 pages) Ponder presents in more or less detail his researches upon hemolysis and the mechanism of simple hemolysins which he believes to fall into certain well defined groups:

1. Acting in a way similar to Saponin.
2. Acting in a way similar to bile salts.
3. Acting in a way similar to acids or alkalis.
4. Producing osmotic changes like hypnotic saline.

The action of each of these members is considered in detail and constructively discussed.

The volume is of great interest to those interested in biologic investigations and well repays its perusal.

The Antisterility Vitamine Fat Soluble E†

THIS monograph is a direct continuation of an earlier series of papers by Dr. Evans in collaboration with Dr. K. S. Bishop and details the studies of the authors upon a specific vitamine discovered in the laboratories of the University of California and found to be essential for the normality of reproduction in mammals.

From extensive studies upon rats the authors are convinced that there is a substance present in the active fractions of their vitamine fat soluble E which is specific for the cure of a specific type of sterility induced by certain purified diets.

Their experiments are fully described and the evidence adduced is of the utmost interest. The report is excellently prepared but it is regrettable that its large size (13 by 10 inches) renders it somewhat unwieldy to handle comfortably.

*The Erythrocyte and the Action of Simple Hemolysins. By Eric Ponder, Lecturer in Physiology, Univ. of Edinburgh. Cloth, 192 pages; 11 figures. Oliver and Boyd, London, England.

†The Antisterility Vitamine Fat Soluble E. By E. H. McL. Evans and G. O. Burr. Paper, XII microphotographic plates, 107 tables, 2 graphs, 3 text figures. Volume viii. University of California Memoirs. University of California Press, Berkeley.

NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

*An Illustrated Guide to the Slit Lamp**

THIS book is founded upon lecture courses given at Oxford University and Trinity College, Dublin and, is intended by the author to serve as an elementary introduction to the technic of the slit-lamp and a summary of the discoveries made with it during the past decade.

That the book is authoritative is suggested by the fact that the author is a pupil of Professor Vogt and served as a demonstrator of the slit-lamp under him.

The volume is an excellent example of the printer's art, the illustrations excellent, and the typography almost devoid of error.

To those utilizing the slit-lamp, this book should be most welcome.

Birth Injuries of the Central Nervous System†

THIS is volume xi of Medicine Monographs which are comprehensive reviews adequately discussing a disease, a certain aspect of a disease, or subjects allowing a better comprehension of disease processes.

Part I (71 pages), by Dr. F. R. Ford of the Johns Hopkins Hospital, discusses cerebral birth injuries. After a short but complete historic review the causes of cerebral birth injuries are covered as stated in the literature and then their results. The author does not believe that it is possible, from the evidence available, to reach any final conclusions about the exact limits of the group of cerebral birth palsies and is of the opinion that cerebral birth injuries are rare rather than common, and that the great mass of infantile palsies can no longer be lightly attributed to faulty obstetric procedures. No final statement can be made as to the relation of intracranial birth injury and chronic hydrocephalus. The common diffuse meningeal hemorrhage not large enough to cause death apparently leaves no residuum in the great majority of cases.

The real cerebral birth injuries are caused by the rarer intracerebral hemorrhages and necroses, depressed fractures with brain laceration and by encapsulated hematomas. All the evidence collected by Ford indicates that the true cerebral birth palsies are represented by congenital hemiplegias, monoplegias, and the assymmetrical and unequal bilateral spastic paralyses. Eight case reports and a list of 136 references accompany this section.

In Part II (44 pages), cord birth injuries are discussed by Crothers and Putnam of the Harvard Medical School. Most of the illustrations are in this part of the book.

The authors conclude that most cases are due to the imposition of the unphysiologic force of traction. Writers of obstetric textbooks, therefore should lay stress upon the nervous system of the fetus as an important factor in successful delivery. Two factors are important in the pathology.

First, cord injuries are due to a type of force never imposed except during labor; the second factor has to do with real but rather vaguely defined differences between fetal and adult tissues.

This book is of real value and appeals, not only to the investigator and pathologist, but to the physician at large, especially to those engaged in the practice of obstetrics.

The Human Cerebrospinal Fluid‡

IN THIS book are collected, in seven sections, thirty-one investigations reported to the Association for Research in Nervous and Mental Disease in 1924.

The contents include: I The Normal Human Cerebrospinal Fluid; II Biological,

*An Illustrated Guide to the Slit Lamp. By T. Harrison Butler, Surgeon, Birminghom Eye Hospital. 144 pages, 158 illustrations, 5 colored plates. Oxford University Press.

†Birth Injuries of the Central Nervous System. By F. R. Ford, B. Crothers, and M. C. Putnam. Cloth 164 pages, 56 figures. William and Wilkins Co., Baltimore.

‡The Human Cerebrospinal Fluid: An Investigation of the Most Recent Advances as Reported by the Association for Research in Nervous and Mental Disease. Cloth, 568 pages, 77 illustrations, 58 tables.

Chemical, and Physical Properties Under Normal and Pathological Conditions; III Pressure Studies; IV Diagnostic Replacement Studies; V Changes in Connection With Central Nervous System Disease; VI Reaction in Extraneural Diseases; and, VII Treatment Through the Cerebrospinal Fluid.

To neurologists and pathologists this volume is of exceeding interest; indeed, it could be read with interest and profit by the physician at large.

*Bacteriology, Blood Work, and Animal Parasitology**

THIS book needs no introduction to the medical or laboratory world at large. It has always been a standard text of indubitable value and probably always will be.

This, the eighth edition, has undergone a thorough revision and is enlarged by about one hundred pages, and not only deserves but will undoubtedly receive as favorable a reception as its predecessors.

As the Kahn test has been adopted as the official Navy serology test for syphilis, the complement fixation methods described in former editions are omitted only Naguchi's anti-human and the Us. P.H.S. methods being described.

As would be expected from so skilled a worker, Stitt emphasizes in italics that only workers properly trained in clinical laboratory procedures should attempt the Kahn test.

There are surprisingly few typographical errors in so voluminous a text.

A Manual of Chemistry†

THIS well-known text was written "to furnish to the student in concise form a clear presentation of the science, an intelligent discussion of the subjects which are of interest to him, and a trustworthy guide to his work in the laboratory."

That it has ably and amply fulfilled these aims the present thirteenth edition furnishes conclusive evidence.

This is a book to be bought, not borrowed.

Every Woman a Nurse‡

THE purpose of this book is to serve as a manual and source of information for nursing societies, technical school classes, Red Cross and Ambulance Societies, and in the home. The book is stated to be a revision and expansion of the author's book on "Home Nursing."

Certain peculiarities of style attract the reader's attention; "This handbook is intended for the use of men and women . . . . who may find themselves in the position that they have to undertake the care of the sick, injured, or wounded." ". . . . see that there is a provision of a W. C.'s etc."

In Chapter XI, "Children's Ailments," one notes paragraphs devoted to the treatment of "growing pains" and, under "headache" a suggestion to "find the cause," which, one might venture to say, might at times be a somewhat difficult matter for "men and women" no matter how earnest their desire to care for the sick.

This book, in the reviewer's opinion contains too little for the nurse and too much for the layman and suggests a type of volume of dubious value, namely, the sort of text intended for the lay treatment of disease in the home.

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*Bacteriology, Blood Work, and Animal Parasitology. By E. R. Stitt, M.D., Surgeon-General, U. S. Navy. Eighth Edition. Cloth, 1 plate and 211 other illustrations containing 683 figures. P. Blakiston's Son & Co.

†A Manual of Chemistry. By W. Simon, M.D. and Daniel Base, Ph.D. Thirteenth Edition, revised by J. C. Krautz, Jr., Sc.D. Professor of Pharmacy, University of Maryland. Cloth, 695 pages, 55 illustrations and 6 colored plates. Lea and Febiger, Philadelphia.

‡Every Woman a Nurse: Health and Nursing Notes. By Edith Newsome S. R. N. Cloth. 204 pages, 31 illustrations, Oxford University Press, New York.

*Normal Midwifery for Midwives and Nurses**

IN THIS book is embodied the lectures delivered by the author to nurses while he was Assistant Master at the Rotunda Hospital.

In the introductory chapter occur these paragraphs which at once assure the reader of a clear, sane, and eminently practical discussion of the subject:

"One thing is absurd, and that is to try and improve matters by increasing the theoretical knowledge of the midwife." (One is strongly tempted to add that this applies with equal force to the nursing curriculum which is almost daily increasing in scope, at least, in America.) "A Sister in the medical world is not expected to discuss the pathology of bronchitis, to percuss a chest, or listen for râles or crepitations, neither should a midwife be expected to know anything about the pathology or treatment of abnormal labor. She should be able to recognize that the labor is abnormal but should not be required to make any diagnosis."

This is a student's text deserving of wide circulation and one which could well be read by many nurses who are, unfortunately, no longer students.

Potassium and Tartrates†

A COMPREHENSIVE review and summary of the literature concerned with the physiologic effects of potassium and tartrates of especial interest to therapeutists, the first part of the book (103 pages) being concerned with the effects of potassium, and the second part (51 pages) discussing tartrates.

The monograph is a comprehensive summary of the subject discussed.

Malarial Psychoses and Neuroses‡

WITH the exception of those whose field of practice has carried them into appropriate localities, it is entirely possible that to many, malaria means "chills and fever."

"It is remarkable," says the author in his Preface, "that although malaria is traceably two thousand five hundred years old, that although it is the oldest disease of which we have any reliable record, that although it is the most widespread disease in the world today, and that although its most characteristic feature, the paroxysm, which through its periodicities gives the varieties of its forms their names, is largely a neurologic phenomenon, there appears to be no comprehensive work dealing with the nervous manifestation of it."

This hiatus he has endeavored to fill in the present volume which is, indeed, a comprehensive and scholarly monograph.

After a brief but clear description of the parasite and the disease, there follows an interesting discussion of malaria in history and its effects upon character and race degeneration.

A well-written section on pathology follows in which are found most of the illustrations. These are mainly microphotographs and colored drawings, well chosen and excellently reproduced. The clinical pathology of the endocrine and other glands is then taken up after which a varied array of malarial psychoses are separately considered.

There are well-written chapters on malaria and alcohol, malaria and surgery, malarial nerve conditions, and malaria in its medicolegal relations, some two hundred illustrative case histories being briefly but clearly summarized.

The author has evidently had a comprehensive experience and speaks with authority.

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*Normal Midwifery for Midwives and Nurses. By G. W. Theobald. Cloth, 258 pages, 28 illustrations. Oxford University Press, New York.

†Potassium and Tartrates: A Review of the Literature on Their Physiological Effects. By R. W. Webster, M.D., Cloth, 168 pages. The Commonwealth Press, Chicago.

‡Malarial Psychoses and Neuroses. By W. K. Anderson, M.D., Recognized Teacher of Clinical Medicine, Glasgow University, etc. Cloth, 395 pages. 18 illustrations, 4 colored plates. Oxford University Press, New York.

The literature of the world has been carefully and intelligently searched and the writings of the ancients have not been neglected; thirty-four pages at the end of the book list the references.

The author is to be congratulated on the completion of a work which will last and the publishers upon its presentation in a worthy and attractive form.

*Poverty, Nutrition and Growth**

THIS volume, Special Report 101 of the British Medical Research Council, is the report of a Committee detailed to study the problems of child life. The work was begun in 1919 under the general supervision of the Scottish Committee for Child Life Investigation and summarizes an enormous amount of data secured by almost incalculable labor.

Part I details the general plan of the work, the populations studied, and the methods employed. The general description of the slum districts covered in these investigations would open the eyes of American Labor disgruntled with its lot. Part II embodies a comparison between the children of the poorer classes in town and country; Part III a consideration of factors generally supposed to influence the nutrition and growth of children; Part IV income and home conditions; Part V diet; Part VI Parental factors; Part VII the children of rural miners; and Part VIII, the children of agricultural labors.

An appendix lists the various forms; etc., used in the investigations. If only for its value in teaching "how the other half lives" this report deserves wide circulation.

The data are of great value and interest to sociologic workers and, indeed, to pediatricians and all interested in human welfare.

The evidence does not substantiate any marked delay in growth as a result of poverty per se but shows that small parents tend to beget small children and that the small size of the town child is an inborn characteristic.

There was no clear indication that nutrition was directly associated with income. A correlation of weight with breast and artificial feeding was manifest up to eight or nine months; after that it was absent.

The most significant and constant finding was the correlation between maternal efficiency and the height and weight of the child. Overcrowded dwellings and an inferior type of mother tended to go hand in hand. This pioneer study, as is usually the case, raises more questions than it answers and points the way to problems for future investigations.

It is impossible, in a short review, to indicate the volume of data contained in the report. It must be read to be grasped and studied to be appreciated.

A Textbook of Biological Chemistry†

THIS book is intended for the elementary student and not for reference and as such amply fulfills the purpose for which it is intended.

*Poverty, Nutrition, and Growth: Studies of Child Life in Cities and Rural Districts of Scotland. By D. N. Paton, M.D., Professor of Physiology, University of Glasgow; and L. Findlay, M.D., Professor of Pediatrics, University of Glasgow. Paper, 333 pages, numerous tables and graphs. H. M. Stationery Office, London.

†A Textbook of Biological Chemistry. By J. B. Sumner, Assistant Professor of Biological Chemistry, Cornell University. Cloth, 283 pages. 4 figures. The Macmillan Co., New York.

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EDITORIALS

Pillars of Salt

AS THE study of scientific medicine progresses one fact assumes pre-eminence. To be of real service to the patient the physician must diagnose the patient's disease before its ravages have laid waste his vital organs. Diseases must be diagnosed in their incipiency, long before there are organic signs and symptoms, and often before there are marked functional disturbances. Our only chance to do this is by a better understanding of the body chemistry which includes the chemistry and functions of the endocrine glands. As the food makes the blood and the blood feeds the cells, it is necessary to know something of the chemistry of food and digestion. What constitutes nutrition, and what is the difference between nutrition and stimulation? How often is a condition of so-called "health" really the mask of stimulation that later destroys the vital organs?

When we try to classify our foods, it is difficult to know where to begin. Organic or colloidal foods; inorganic or crystalloidal foods; raw foods which may come under the heading of hydrophile colloids and cooked foods which

may come under the heading of hydrophobe colloids and inert foods which do no more than serve as ballast. Then there are substances which in small doses stimulate and poison insidiously and in larger doses poison and cause deterioration of vital organs. Inasmuch as the physiologists believe that most foods should be of a colloidal or organic nature and that the chemistry of digestion is a problem of colloid chemistry, our attention is drawn to a crystalloidal substance that is now being consumed in great quantities. Its popularity has even been enhanced of late because we can now obtain it in the "iodized" state. I refer to sodium chloride, inorganic.

Long ago it was observed that in certain states of organic deterioration salt seemed to aggravate the condition. Now we know that it interferes with the elimination of certain waste products of metabolism, whereas in earlier days it was noted that the nephritic patient grew more edematous, the "Salt Rheum" increased in rheumatism or eczematous states. Haig showed that it interfered with the elimination of uric acid products. Later it was shown that in animals such as dogs and in birds such as chickens where a good deal of the nitrogen is eliminated as uric acid, the result of feeding salt, even in very small quantities, was death. Autopsy showed the liver and kidneys studded with uric acid concretions.

Yet, what is the most common argument for salt eating? That animals like it, need it, even travel miles to so-called "salt licks" to get it. How do we know that these animals are not mineral starved and lick salt as a poor substitute for browsing leaves and twigs? Even when we give the horse plenty of salt he still chews the bark off trees, gnaws his manger boards and rasps the telephone poles. Does the fact that animals like salt mean that they need it any more than that we human beings need strong coffee because we like it? Introduce a horse to sugar and give him his choice of a sugar mash or a salt mash. He will gorge himself with the sugar mash and ignore the salt mixture. Does that mean that horses *need* sugar? At best the argument is most flimsy, and would not have a leg to stand on were it not for the fact that salt is a stimulant. It makes us feel good by elevating the blood pressure a little and stimulating the adrenal glands, with the result that the cheeks and ears glow. It also brings a happier mental state through a feeling of warmth and good health. We say that salt is necessary for life. But is it? Benjamin Rush found the American Indians as healthy as Stefansson found the Esquimaux or as Bartholomew found the Chinese of the Interior, none of whom ever ate salt.

In the days of our forefathers salt solution was used as an embalming fluid. The ancient Egyptians used oils, spices and salt in their mummy wrappings. Today we mummify the living with salad dressings made of mineral oils, spices and salt. You can see any number of these mummies walking the streets. The dry skin, shrunken bodies and white hair bespeak the hardened livers and sclerotic kidneys. I often wonder why it is necessary to embalm such bodies after they *are* dead. They are already "pickled to the gills."

It is possible for a person to eliminate salt quite rapidly through channels such as the skin and kidneys. As long as the body is strong, the resist-

ance good and the glands of internal secretion adequate, not much salt is retained. But when the channels of elimination are inadequate salt retention, with its attending harmful consequences, results and the liver or kidneys or skin, or all three, may show at first functional derangement followed by organic destruction. Albumin, casts, red blood cells and leucocytes in the urine may be signs of so-called Bright's disease. If they are, may not such extensive kidney destruction be the third stage of salt poisoning? The second stage may be the transient albuminuria after moderate exercise or an unusually heavy meal; the first stage the excess of NaCl in the urine, which causes no signs or symptoms and which occurs when the patient feels good and thinks he is in a healthy state. In the third stage the kidneys are so impaired, that salt elimination is greatly interfered with. This is a very poor time to think that by restricting salt in the diet we can accomplish very much for the patient. Somewhere along the line there was a point where the salt began to be dangerous. The railroads have found that the best way to eliminate dangerous crossings is to eliminate crossings. Have the people cross the tracks overhead or through subways. In other words, "stay off the tracks." The eating of inorganic salt is a bad habit. Why not let the plants organize NaCl into a colloid form, in their leaves and fruits and roots and stems, and eat it that way? The urine and sweat never show an excess when it is consumed in this form.

The great Mackenzie wrote, "The first appearance of disease in the human body is invariably insidious, with little disturbance of the economy and no visible signs of its presence. By and by the patient becomes conscious that all is not well with him; there is a loss of that feeling of well-being which accompanies the healthy state. Disagreeable sensations arise, at first vague, but later becoming more definite and these may become so urgent that he seeks advice. Still no evident sign of disease may be perceived on the most careful examination. By and by the disease, being situated in some organ or tissue, changes the constitution of that part, so that its presence is now recognized by a physical sign, when the clinical methods usually employed reveal its character."

It is only by careful chemical examination of secretions such as mucus, tears, gastric juice, urine, joint fluids, spinal fluid, and blood that we can arrive at early diagnoses of salt poisoning. Our figures for the normal are all too high, since most of the so-called normal cases were early cases of salt retention. Again to quote Mackenzie: "There are evidences which would surely indicate the nature of the disease in its earliest stages, were we capable of detecting them." If we are ever to be able to detect these evidences we must look for them as chemical pathologists.

—*H. B. (P. G. W.)*

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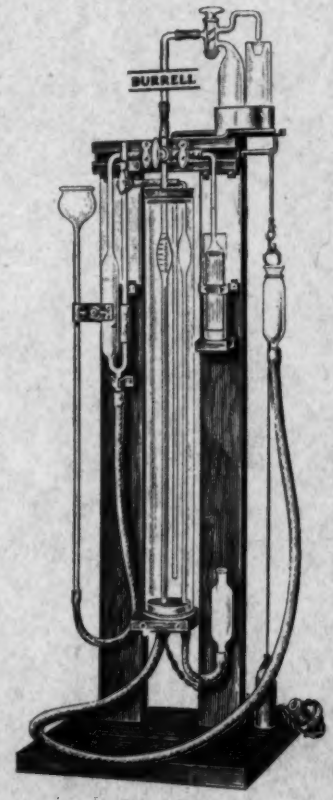

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